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Discovery and Verification of Quantitative Trait Loci (QTLs) for Seminal Root Traits and Insights Into Root to Shoot Tradeoffs in Hexaploid Wheat (*Triticum aestivum L.*)

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

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by

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ABSTRACT OF THE DISSERTATION

Discovery and Verification of Quantitative Trait Loci (QTL) for Seminal Root Traits and Insights Into Root to Shoot Tradeoffs in Hexaploid Wheat (*Triticum aestivum L.*)

by

Christopher Earl Hohn

Doctor of Philosophy, Graduate Program in Plant Biology
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Dr. Adam J. Lukaszewski, Chairperson

Wheat is among the top three cereal crops with over ca. 600 million tons being harvested annually. In terms of its range of cultivation no other crop can rival wheat. It was first cultivated over 10,000 years ago as humans shifted from hunting and gathering to settled agriculture. Since then wheat has seen more than a threefold increase in grain yield and makes up ca. 20% of the human diet. Today climate change and increased incidence of drought in areas a wheat production negatively impact grain yield. This has prompted interest in studying root system traits and how those traits may improve drought tolerance. For these reasons, the research in this dissertation was aimed at identifying quantitative trait loci (QTLs) and allelic variation for root system traits while also gaining an understanding of root and shoot relationships. To accomplish this three integrated mapping populations of bread wheat were created and sets of unique

experiments were conducted. Significant variation for root system traits was observed in all three populations and QTLs were identified and verified for some of these traits. One major QTL for seminal root angle on chromosome arm 2DS was verified in two of the three mapping populations. Additionally, we were able to draw some general conclusions about the relationship between root and shoot biomass within the materials we tested. Using over ca. 6,000 data points we observed that as root biomass continues to increase beyond a certain threshold it negatively impacts grain yields and shoot biomass. However, in individual cultivars this relationship may be entirely different, with root biomass increasing proportionately to increasing grain yields without any observable threshold. When testing for allelic variation at a locus thought to control root biomass on rye chromosome arm 1RS we were unable to identify any significant differences between sources of the 1RS translocation. In a similar study testing for allelic variation for a locus on wheat chromosome arm 1BS thought to control root system plasticity in response to drought we were also unable to identify any significant difference between 1B substitution lines in a common genetic background of cv. Pavon 76.

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General Introduction

Since its domestication, wheat yields have increased at least tenfold, and its cultivation has spread to essentially every continent. This feat is more amazing considering that selection has always been exerted for only the above ground parts of the plant, notwithstanding the fact that plants depend on their roots for a range of tasks from supplying water and nutrients, to anchorage. Of course, selection for the above ground parts must have affected the root system as well, but only indirectly and not necessarily in the most desirable way. Some reports show that the Green Revolution wheats, because of their dwarfed phenotype along with a reliance on irrigation and high fertilizer levels, had their root system seriously reduced (Waines and Ehdaie 2007). The time has come to find out what impacts have come about from neglecting the root system and determine if there are root character traits that can improve yields further.

It is being argued (Dennison, 2012) that many crops have reached their physiological limits of productivity and only extra efforts can keep them increasing. Improvement of root systems may be the step necessary to increase yield potential. Most areas of wheat cultivation are under rain-fed conditions making water an essential and limiting resource. It is known that rainfall patterns have become less predictable and climate change is highlighting the necessity to develop adapted cultivars. I believe now is the time to devote roots the attention they deserve and many others are thinking the same way. There have been a number of studies published in recent years regarding some aspects of wheat root system genetics (Bai et al., 2013; Christopher et. al., 2013, Hamada

et al., 2012; Liu et al., 2013; Zhang et al., 2014). However, despite these publications there are no markers for these traits available to breeding programs and no genes have been identified.

It was suggested by Manschadi et. al. (2008) that selection for root growth angle and the number of seminal roots may help to identify genotypes better suited for drought conditions. In addition, it is generally thought that increased root biomass will reduce yield losses in limited-water environments. If this is the case we should be able to locate major loci that are responsible for these character traits and associate them with higher drought tolerance. As will be demonstrated in the following chapters of this dissertation, the development of three mapping populations comprised of three parents with significant differences in root characteristics could be a beneficial tool in the progress toward a better understanding of root system traits in wheat.

Rationale

Wheat is among the top three cereal crops grown worldwide with an unrivalled range of cultivation regions which exceeds all other cereals in total area and production (Shewry, P.R. 2009). Most of these areas of cultivation are under rain-fed conditions making it necessary for cultivars to be productive even under poor conditions. Weaver (1926) provided general characteristics of root systems in many crops; including wheat, however, since then very few genes associated with root traits have been identified in crops besides rice. These facts make wheat an ideal choice for elucidating the genetics of root characteristics lending to improved drought tolerance.

A general approach to breeding for drought tolerance may prove to be too broad. Elucidating individual components of drought tolerance may be more effective since drought tolerance is a quantitative trait with a multifaceted phenotype which complicates breeding efforts (Fleury, et. al. 2010). It is likely that drought tolerance is associated with many different phenotypes; the root system may well be one of the most important ones. To simplify breeding efforts, quantitative traits such as drought tolerance need to be physically and genetically dissected to determine individual phenotypic contributions to the overall expression of the character. This dissection will ultimately enhance the efficiency of marker-assisted breeding strategies (Mir et. al. 2012). Once the major mechanisms of drought tolerance are understood and the genetic controls dissected, elite cultivars can be produced through pyramiding of those traits.

Although there have been several studies that have identified quantitative trait loci (QTLs) for root traits in wheat, given the range of environments and germplasm diversity, it will take more than a few studies, no matter how well executed, to work out the issue to the point of practical recommendation and/or breeding. Likely, it will take multiple projects to verify findings and establish methods to be applied in breeding programs. Here characterization of the root system in a set of wheat hybrids is used to identify some associations between individual traits and associate relevant loci with DNA sequence-based markers. Additionally, the relationship of shoot and root traits is considered to help draw some conclusions for future research to focus upon.

Experimental Plan

The research plan is based on two hypotheses: first, a narrow seminal root angle in wheat is important for adaptation to drought by allowing deeper rooting and better water acquisition, and second, the relationship of root to shoot biomass will determine the overall performance of wheat under stressed conditions. It is proposed to concentrate on these traits because they are major keys to root distribution in the soil as well as growth dynamics of seminal roots. To accomplish this project, a set of doubled haploid (DH) mapping populations has been created and will be implemented.

The primary goal in developing mapping populations is to identify loci that affect the expression of a trait within that population. Estimation of the magnitude of the genetic effect is also essential to these types of studies. In 1998 Beavis demonstrated that in populations numbering 100 progeny, the quantitative trait locus (QTL) effects were greatly overestimated, in populations with 500 progeny the QTL effects were slightly overestimated while populations with 1000 individuals produced estimates close to the actual magnitude of QTL effects. That study highlighted the necessity for larger populations and the need for verification of QTL across populations. In another study by Stange et al. (2013), high density genotyping was shown to improve QTL localization, effect estimates, and resolve closely linked QTL.

Three spring wheats, Foisy, Sonora, and Chiddam Blanc de Mars (CBdeM), with significant differences in root architecture, seminal root characteristics, and root biomass were used to create three mapping populations. Crosses were made in such a way that each parent is present in two of the three populations: Sonora x CBdeM (abbreviated SC),

Foisy x Sonora (SF), CBdeM x Foisy (CF). For each population ca. 150 lines were genotyped giving an effective population size of 300 lines for each parent. This crossing scheme provides for instant verification of QTLs across populations and narrowing in on the gene(s) responsible for traits of interest. It is expected that reliable QTLs will be identified in at least two of three populations provided that parents are heterogeneous for the alleles. High density genotyping of these three populations was completed using the Illumina iSelect 90K SNP assay. SNP calls were made using the Polyploid Clustering Module of Genome Studio (Illumina) and linkage maps were created using JoinMap4 (Kyazma). Phenotyping for basic morphological traits such as plant height, awns and such physiological traits as flowering time, grain yield, etc., will permit associations with specific genomic regions and this in turn will verify map quality and provide general reference. These populations along with the linkage maps are publically available.

Recently, QTL analysis for root traits has gained an increasing interest. Previously most research has been focused in rice and maize. Weaver (1926) was one of the first to detail various root morphology of different crops and look at distribution of roots in the soil. Root architecture is determined by growth angle, total root length, and lateral branching. In 1993, Oyanagi et al. hybridized a cultivar of wheat with a wide angle and one with a narrow angle. The F1 hybrid showed an angle equivalent to that of the parent with the wide angle, and the distribution among the F2 was bimodal, with most plants having wide values and a small group giving narrow values. Thus, it was assumed that wide root angle was controlled by a single gene. Drawing ideas from maize research, Oyanagi (1994) suggested that gravitropic responses of roots would be the easiest to use

for estimations of wheat root distribution in the soil. So far, no gene for this character has been identified in wheat. However, a gene was identified and cloned in rice (DRO1), which was shown to control the gravitropic response of roots (Uga et al. 2013).

Previous QTL analyses for seminal root angle have been conducted on different wheat populations (Hamada et al., 2012; Christopher et al., 2013). Both studies used a limited number of markers, 276 SSR and 841 DArT, respectively, and dealt with single populations. The population used by Hamada et al. consisted of 103 F1 derived doubled haploids and the population of Christopher et al. consisted of 77 F1 derived doubled haploids and 107 BC1 derived doubled haploids. No QTL for root angles were detected by Hamada et al., nor were other QTLs for other root traits similar across both studies. A potential problem could be a lack of large phenotypic difference between the parents used; Christopher et al. report that one parent had a root angle of 39.6 degrees and the other had an angle of 41.3 degrees. As noted by Tanksley (1993), the greater the phenotypic difference between two individuals used in deriving a segregating population the greater the chances are of detecting significant QTL. Hamada et al. (2012) did not report the average angle of either parent used to derive their mapping population. These examples show the need for verification of possible QTLs and further analysis with larger populations and a higher density of markers.

Many root morphological traits are regulated by a number of small-effect loci that interact with the environment. This becomes very apparent when conducting experiments testing root biomass and length. In fact, in many cases the amount of plasticity due to the environment creates such large errors that it is often difficult to measure such traits

accurately. For these reasons, Dorlodot et al. (2007), suggested that process-based traits such as growth rate, branching frequency and tropism should be studied as opposed to 'static traits' such as length, mass, and volume. That being said, biomass can be an important factor, along with other root characters, that allows for improved drought tolerance.

Larger root systems and deeper roots in the soil profile is an obvious strategy used by plants to acquire available water when rainfall is limited. As water becomes less available at the surface, crops not adapted to reach the water available lower in the soil profile suffer. It has been suggested that roots targeting water acquisition deep in the soil profile may be especially important for smaller statured plants such as rice, wheat, and common bean (Comas et al., 2013). For these reasons efforts need to be made to develop cultivars better adapted to limited water, however, understanding the relationship between shoot and roots will be essential for any progress. Although plants with larger and deeper root systems may be able to explore more of the soil profile excessive allocation of resources to root growth may have a negative impact upon grain yields when water is more accessible or when compared to a lean root system that reaches deeper into the soil profile. Recently Lynch (2013) proposed an ideotype for maize roots that would optimize water and nitrogen acquisition, which may be relevant to other cereal root systems. This ideotype includes narrow seminal root angles with abundant lateral branching while maintaining an overall lean root system. The idea is that the root system cannot cost the plant too much when it is already under stressful conditions. Maintaining

a large and costly root system could put strains on carbon and resource allocation causing reductions is yield.

Not only will it be important to understand the relationship of roots and shoots but identifying loci controlling the two will help to understand the issues at hand as well. The only example in wheat, as far as I know of, QTL mapping for root biomass was done by Sharma et al., 2011. They mapped QTLs for different root traits, including that for root biomass, on the short arm of rye chromosome 1R in bread wheat using 1RS-1BS recombinant lines. Another example of identifying chromosome regions influencing root biomass comes from Ehdaie and Waines (1997). In this paper they identified genomic regions for responsible for various traits by using telosomic lines in bread wheat. Beyond these two studies there is still a need for verification and identification of genes controlling root biomass.

Broader Impacts and Future Perspectives

I hope that this research will provide an important step toward the understanding of genes responsible for root characteristics in wheat, and perhaps lead to the development of breeding tools for better drought tolerance and clear clues on the design of experiments addressing very specific question. I believe this project will ultimately lead to cloning of the responsible genes in the future. An increased knowledge of the genetics of root adaptations to drought will lead to deeper research on the topic. Results have been or will be shared through publications, presentations, and discussion forums enabling progressive future research to help agriculture remain productive in a changing

environment. Each objective in this project will provide a foundation for future research. Being able to identify relationships between shoots and root architecture traits will enable efforts to further understand the genes controlling these traits. Information obtained from these projects could be applied in other crops such as rice, barley, rye, and maize as well. Not to be ignored is the fact that wheat is one of the top three staple crops in the world with the least information about these topics making the data presented here valuable to our understanding.

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Chapter 1

Three Integrated Mapping Populations for Studying Root Architecture Traits in Bread Wheat

Abstract

Persistent predictions of climate change and increased drought has led to an increased interest in crop root systems. Drought tolerance is a complex trait and most root system traits are heavily influenced by the environment. Root system traits are quantitatively controlled and their plasticity makes them difficult to study. This calls for tools such as specifically designed mapping populations. Here three integrated mapping populations of doubled haploids were developed with a built in system for verification of quantitative trait loci (QTLs) across genetic backgrounds. The three parents, Sonora, Foisy, and Chiddam Blanc de Mars, are “traditional cultivars” selected from landraces each being hundreds of years old and could be considered landraces themselves. They were chosen for their contrasting phenotypes including drought tolerance and root traits. The populations were genotyped using the 90K Illumina SNP array and high marker density genetic linkage maps were generated and verified by mapping some important agronomic traits. Two major QTLs for awn type were mapped to chromosomes 5A and 6B, five major QTLs responsible for flowering time were located on chromosomes 2D, 5A, 5B, and 5D, and two major QTLs for hybrid necrosis were mapped to chromosomes 2B and 5B. These exercises show that the quality of the linkage maps can be trusted. It

has also been demonstrated that the design and relationships of these populations allow for instant verification of traits of interest when all three are used together in evaluations. This new resource is available to those interested in genetic dissection of root traits, and should become a valuable tool for many related studies.

Introduction

Plant root systems have never received as much attention as the above ground portions of plants. This is understandable given the relative ease of studying shoots and leaves versus roots. It is, however, well recognized that roots are vital to a plants livelihood and certainly are no less important than the above-ground parts. As agriculture is facing changes in climate patterns and increased incidence of drought, roots are gaining more attention. In recent years, more articles have been published with a focus on root systems in crops than there has ever been since Weaver's (1926; 1927) groundwork on the subject. Of the top three cereal crops grown worldwide, rice and maize have received most of the attention for root system genetics (Ahmadi et al. 2014; Mai et al. 2014; Hochholdinger and Tuberosa 2009). More recently, wheat root system genetics has also seen an increase in attention with hopes of improving drought tolerance. This makes sense in that wheat makes up nearly 20% of the world's caloric intake each year (FAOSTAT 2014). However, when compared to rice and even maize the wheat genome is much more complex and makes quantitative studies that much more challenging.

Not only is drought tolerance a quantitative trait (McWilliam 1989) but most root system traits are highly plastic and also quantitatively inherited (Dorlodot et al. 2007; Cooper et al. 2009). These facts make studying root systems and their relationship to drought tolerance a fairly daunting task. To simplify the process it has been suggested that drought-tolerance traits should be dissected using genomic tools such as quantitative trait locus (QTL) mapping and microarrays (Fleury et al. 2010). It is likely that certain

root system traits are critical to improving drought tolerance in wheat and thus should be studied in more detail (Pinto 2015; Placido et al. 2013; Reynolds et al. 2007). Current research on rice and maize has shown that indeed roots are important factors in reducing yield losses under water-limited conditions in the field (Uga et al. 2013; Lynch 2013).

Given that the root system of wheat has only recently gained interest, only a limited number of mapping populations have been developed specifically for this purpose. All existing populations have the disadvantage of not offering any quick verification of quantitative trait loci (QTLs) in different genetic backgrounds. For these reasons three integrated mapping populations of doubled haploids in hexaploid bread wheat were developed. The way in which these populations were developed allows for the simultaneous testing of QTLs in different genetic backgrounds. This provides instant verification of QTLs across genetic backgrounds as well as environments when all populations are included in experimental trials.

Materials and Methods

Mapping population parents

In 2009 and 2010 Waines et al. (2012) evaluated 17 spring wheat landraces and modern cultivars for root biomass. Their results were used to select the appropriate parents for the three mapping populations. Cultivars Sonora, Foisy, and Chiddam Blanc de Mars (CBdeM) were selected. The primary criterion was the total root biomass with Sonora ranked among the highest, Foisy was intermediate, and CBdeM had a low total

root biomass. Additionally, the three also have other contrasting phenotypes for traits such as drought tolerance, plant height, days to heading, awn type, and seminal root angle. These parents are what could be considered as "traditional cultivars" in that they were all selected from landraces. Sonora was selected from a landrace in Durango, Mexico and is known for good drought tolerance but its height makes it susceptible to lodging. Cv. Foisy was selected by Mr. Foisy in Oregon in 1865 and typically yields more than CBdeM and Sonora. CBdeM originates from Ville de Paris, France, and was selected from an English landrace. None of these cultivars have a place in commercial agriculture today but still are grown by traditional or artisan farmers as so called heirloom varieties of wheat for bread making. More information about the parents can be found on the UC Davis small grains web page in the 2011 California cultivar descriptions publication (http://smallgrains.ucdavis.edu).

Crosses were made in a triangular manner to form a set of "nested" mapping populations with any given two populations having a single parent in common so that we get the populations Sonora x CBdeM (abbreviated as SC), Sonora x Foisy (SF), and CBdeM x Foisy (CF) (Figure 1.1). This design provides a built in system for verification of QTLs across populations and genetic backgrounds.

Doubled haploid (DH) development

F1 seeds from the three crosses were sent to Heartland Plant Innovations (HPI) at Kansas State University for the production of DHs. Wide hybridization methods (wheat x maize) similar to those first described by Laurie and Bennett (1986) were used to induce

haploids. No details were given of the HPI protocol used but a typical procedure includes emasculation of the F1 wheat spikes and pollination with maize pollen. Post pollination spikes are typically treated with 2,4-D or GA_3 to promote healthy embryo growth and embryos are rescued onto a culture medium. Finally, colchicine is applied to haploid plants to generate doubled haploids (DH). The harvested seed was then sent to us at the University of California, Riverside.

Population characterization

For each of the three populations ca. 200 lines were planted on July 11th, 2013 in an air-conditioned greenhouse on the UC campus in Riverside, California, in one gallon pots with two plants per pot. These were used for seed increase, leaf tissue for DNA extraction, and for phenotyping of simple traits. Doubled haploid plants in each pair were compared and expected to be identical, however, not all were and any lines with clear differences between the two plants were discarded. A second seed increase was planted on April 4th, 2014 in a similar manner and was also used to collect phenotype data. During this increase plants were grown under 18 hour days with supplemental lighting.

In 2015 a two-location field trial was established. Experiments were planted in October 2015 and harvested by May 2015. The two locations were the University of California, Riverside Agricultural Experiment Station (UCR) in Riverside, California, and at the Coachella Valley Agricultural Research Station (CVARS). Experiments were set up in randomized augmented designs with three check varieties replicated in each block. The check varieties were Blanca Grande 515, Summit 515, and Cal Rojo. There

were 32 blocks per treatment per location with 16 plots per block. Additional "blank" plots of Summit 515 were planted to make blocks square but were not included in the analysis. Each plot consisted of six rows spaced 20cm apart and 122cm long planted at a density of 560 seeds per plot. Each location had two treatments, one well-irrigated and the other which received limited irrigation after 60% of the genotypes were booting. The well-irrigated treatment received water as needed based upon soil moisture and plant indications. For the limited irrigation treatment water was withheld until plots showed moderate to severe wilting at which point they were irrigated to prevent death. All other cultural practices were standard for wheat production in the area. The R statistical package "lmerTest" was used to obtain the predicted mean values for all traits evaluated which included; days to heading, plant height, yield/m^2, and 1000 grain weight (TGW).

Genotyping

Ca. 100 mg of fresh leaf tissue was collected from each genotype into 2 ml microcentrifuge tubes with conical screw caps and O-rings (Fisher Scientific, Waltham, MA, USA) and dried over silica gel (S684-212 6-12 mesh, grad 40 desiccant Fisher Scientific, Waltham, MA, USA) under reduced air pressure in desiccators. After three days, samples were pulverized for 60 s with two 3.2 mm chrome steel beads (BioSpec Products, Inc., Bartlesville, OK, USA) and 150 mg of S25-500 sand (Fisher Scientific, Waltham, MA, USA) in a FastPrep-24 instrument (MP Biomedicals, LLC, Santa Ana, CA, USA) and DNA was extracted using the protocol listed at the Diversity Arrays Technology website (http://www.diversityarrays.com). Extracted DNA was diluted to

50ng/uL in a 20uL volume with the TE buffer and loaded onto 96-well plates for genotyping with the Illumina iSelect 90K SNP array. Genotyping was done at the USDA-ARS Cereals Crop Research Unit in Fargo, ND under the kind supervision of Dr. Shiaoman Chao.

SNP calls were made using the Polyploid Module of GenomeStudio (Illumina). Akhunov et al. (2009), Cavanagh et al. (2013) and Wang et al. (2014) have shown the complexity of genotyping polyploid wheat arising from the presence of homoeologous and paralogous gene copies in the genomes of tetraploid and hexaploid wheat. For these reasons all 81,587 markers required verification of proper calling and poorly separated clusters were called manually.

Linkage map construction

SNP calls from GenomeStudio were converted into "A" (maternal parent) and "B" (paternal parent) genotypes by comparison against parental scores for each population. Markers that were polymorphic between parents for each population were imported to JoinMap 4.1 (http://kyazma.com) (Stam 1993) and used to construct linkage maps. Chromosome and marker index number were used to name markers previously mapped by Wang et al. (2014), for example 5A_6716, and markers that were not previously mapped were named using an underscore and the marker index number with no chromosome indication. Identical individuals were excluded from the genotypes used to construct linkage maps and likely arose as artifacts from the DH procedure or were a result of labeling errors. Also, individuals with greater than 10% missing data for marker

calls were excluded. Initially linkage groups were generated based upon markers mapped by Wang et al. (2014) using eight doubled-haploid mapping populations. This was done on a chromosome by chromosome basis including only markers mapped to a given chromosome. Identical markers for the given chromosome were removed prior to mapping. Groupings were made using the default calculation settings for independence LOD and linkage groups were mapped using the default settings for the maximum likelihood algorithm.

For linkage groups that failed to generate maps or lacked a sufficient number of markers, additional markers were added from the unmapped pool of SNPs. To do this, all unmapped markers were selected along with the mapped markers for the given chromosome and then the steps listed above were repeated to give new linkage groups with a more suitable number of markers. These newly added markers were then BLASTed against the wheat arm survey sequence to verify their correct linkage group assignment.

Quantitative trait loci (QTLs) mapping

Phenotypic data for awn type, days to heading, and plant height collected during 2013, 2014 greenhouse evaluations and 2015 field evaluations were used to map QTLs by the software package ICImapping (http://www.isbreeding.net) (Li et al. 2007). For greenhouse data, the linkage maps and mean value for two plants of each doubled haploid line were used to map QTLs and for field data the predicted mean values for each genotype were used. The composite interval mapping method with a step of 1 cM was

used and the threshold for QTLs detection was determined using 1000 permutations at $\alpha = 0.05$.

Results and discussion

Phenotypic characterization

Originally, populations SC, SF, and CF consisted of 257, 244, and 214 lines, respectively. During the 2013 seed increase populations were assessed for vernalization requirement, hybrid necrosis, and uniformity. Of the 200 lines planted for SC, SF, and CF population about 1, 2, and 7%, respectively, showed what appeared to be segregation but could have arisen from multiple unknown reasons. Winter growth habit appeared in 18.5, 7.5, and 1.5% of the SF, CF and SC populations, respectively. This was despite the fact that all three parents are spring wheats and require no vernalization. The appearance of winter growth habit may reflect some combination of recessive alleles of vernalization genes (Stelmakh 1987). Hybrid necrosis was rated on a scale of 1 to 10 with 1 being minor and 10 being lethal. Hybrid necrosis was fairly prevalent in SF with 31% of the genotypes showing some level of the phenotype; the CF population had 25% of the genotypes showing some level of hybrid necrosis and the SC population only 6.5%. Any genotype with a score greater than 3 was excluded from genotyping. Because of possible contamination (clear phenotypic differences between two plants in each pot), sterility and with some lines showing winter growth habit and/or hybrid necrosis, populations were reduced in size. Additionally, population sizes had to be limited to genotyping of 150

lines for several reasons, from the cost/practicality issue to future experiment manageability. However, all non-genotyped lines are preserved and can be accessed, if needed.

During the 2014 greenhouse evaluations the remaining 150 lines for each population were characterized for vernalization requirement, days to heading, plant height, and awn type (Figure 1.2). For field trials populations were reduced to 133, 121, and 115 lines for SC, SF, and CF respectively due to winter habit or late flowering of some lines and lack of seed for others. During the 2015 field evaluations populations were characterized for days to heading and plant height, awn type and 1000 grain weight (TGW). However, not all field data could be analyzed and/or were unreliable, thus distributions of trait values in the field for days to heading, plant height at Coachella Valley are shown in Figure 1.3 for (a) SC, (b) SF, and (c) CF.

Genetic Maps

After excluding identical lines and lines with >10% genotyping error, populations were reduced to 146, 141, and 128 lines for SC, SF, and CF respectively. Linkage maps were created using 1187, 1153, and 952 polymorphic markers for the SC, SF, and CF populations respectively. The low polymorphism seen here is due to the ascertainment bias created when selecting lines for the SNP discovery panel which included mainly cultivars (Wang et al. 2014). The 21 linkage groups had an average of 56.0, 53.9, and 44.9 markers each for SC, SF and CF, respectively. However, there were clear differences in marker coverage in different genomes. In three populations combined, the

A genome groups have an average of 60.7 markers, the B genome has an average of 70.1 markers, and the D genome has the fewest makers, at 24.0. Low representation of D-genome markers is a well-known fact. This is likely due to the recent (ca. 8,500-10,000 years ago) occurrence of hexaploid wheat (Akhunov et al. 2010). Perhaps this could have been improved by including D genome progenitors or synthetic wheats in the SNP discovery panel. The average numbers of markers per linkage group in the D genome include additional, previously unmapped markers added for these populations. Through a great deal of manual marker calling and verification a fair amount of unmapped markers were added to each population. Additional 7, 39, 8, 5, and 41 previously unmapped markers were added to the 2D, 3D, 4D, 5D, and 6D linkage groups in SF, respectively. For CF, linkage groups 3D, 4D, 6D, and 7D gained additional 5, 5, 6, and 3 markers respectively. In the SC, the D genome linkage groups had sufficient numbers of mapped markers for five chromosomes; for 3D and 4D 70 and 8 additional markers were mapped, respectively. After accounting for identical unmapped markers added to each linkage group across populations, the total number of markers added to 2D, 3D, 4D, 5D, 6D, and 7D are 7, 92, 16, 5, 47, and 3 respectively. On average linkage groups were 199.87, 193.35, and 125.49 cM in length for the A, B, and D genomes, respectively. The average linkage group length was 172.90 and the total genetic distance of the genome was 3,630.96 cM. This gives an average marker spacing of 3.29, 2.75, and 5.24 cM for the A, B, and D genomes, respectively. Figures 1.4, 1.5, and 1.6 show the linkage maps for populations SC, SF, and CF respectively.

Association of agronomic traits with genome region (QTLs)

Using phenotypic data collected during the greenhouse evaluations (2013, 2014) and the field evaluations (2015) QTLs were mapped to verify the quality of the genetic maps and provide some basic genetic information about the three populations. Table 1.1 summarizes regions that were mapped in the (a) SC, (b) SF, and (c) CF populations for awn type (AWN), days to heading (DTH), plant height (PLTH), and hybrid necrosis (HNEC). Only the regions consistent through multiple years are discussed. Table 1.2 demonstrates how QTLs can be verified within and between populations by using awn type QTLs as an example.

Awn type

Inheritance of awn type, or 'awnedness', in wheat has been well studied and the genetic controls of this trait have thus been worked out to some detail. For that reason awn type makes for a suitable trait to test the quality of the genetic maps generated from these populations. Awn type is a relatively simple trait with three dominant inhibitors known: *Hd* (hooded), *B1* and *B2* (tipped 1 and 2). Wheats homozygous for recessive alleles at all three loci, *hd*, *b1* and *b2* are fully awned; those with *Hd*, *B1* or *B2* are awnless (McIntosh et al. 1998). Chromosome locations of these three loci were originally identified using aneuploid lines with *Hd* located on the short arm of chromosome 4A (4AS) (Sears 1954, Rao 1981); *B1* on the long arm of chromosome 5A (5AL) (Sears 1954); and *B2* on the long arm of 6B (6BL) (Sears 1954; 1966).

For simplicity of the exercise lines were classified into two groups: awned and awnless. Two genome regions were consistently identified that explained 23.76 – 92.67% of the phenotypic variation within the populations. The first locus was consistently identified in the SF and CF populations on chromosome arm 5AL. The QTL in the SF population covered a 2.2 cM region between the markers 5A_9620 and 5A_6716 with the peak around 266 cM explaining 36-39% of the phenotypic variation across all years and environments. In the CF population, the QTL covers a 3.2 cM region between 5A_9620 and 5A_6716 with the peak around 169 cM. The QTL explains 31-93% of the phenotypic variation observed in the population across all years and environments. This QTL shares the same two markers in common with the QTL identified in the SF population. Additionally, Mackay et al. (2014) mapped the same QTL using a wheat MAGIC population and verified it using an association mapping population. They identified the marker BobWhite_c8266_227 as being the closest linked to the QTL which in these populations mapped to the same genetic location as 5A_6716 identified here.

The second QTL was consistently identified in the SC and SF populations as being on the chromosome arm 6BL. In the SC population the QTL covers a 4.2 cM region between markers 6B_606 and 6B_1614 with the peak around 103 cM. It explains 62-73% of the phenotypic variation for this population across all three years and two environments. In the SF population the QTL covered a 0.71 cM region in 2013 and 2014, and a 2.1cM region in 2015 with the peak being around 79 and 80 cM respectively. This QTL explains 23-29% of the phenotypic variation in the population. In 2013 and 2014 the QTL was between 6B_45514 and 6B_606, however, in 2015 it shifted by a couple

markers to 6B_68633 and 6B_84 covered a larger region. However, its peak was still near the same point and the two markers associated with the phenotype in 2013 and 2014 were present in the 2015 region. The QTL shares the 6B_606 marker in common with that identified in the SC population (Table 1.2).

These results indicate that Sonora carries the dominant allele for *B2* on 6BL and that Foisy has the dominant allele for *B1* on chromosome arm 5AL. Since CBdeM is fully awned it must have the *hd b1 b2* genotype.

Days to heading

The trait 'heading date' or 'days to heading" in wheat is determined by several factors, including vernalization requirement controlled by the *Vrn* genes (they control the spring and winter growth habits), the photoperiod genes (*Ppd*) play a role in determining the sensitivity to photoperiodism and the Earliness *per se* (*Eps*) genes are responsible for controlling flowering time regardless of photoperiod. In the three populations studied here, five major QTLs were found responsible for the heading date character, located on chromosomes 2D, 5A, 5B, and 5D.

Two consistent QTLs on chromosome 2D were identified in the SC and CF populations in 2015. In the SC population the QTL covers a 0.67 cM region with its peak around 112 cM between markers 2Dx_32130 and 2Dx_79444. This QTL explains 18.43% of the phenotypic variation seen in the population and has an average additive effect of 7.69 days. In the CF population the QTL covered a 5.8 cM region with a peak around 47 cM between markers 2Dx_7001 and 2Dx_13208. This QTL explains 70.06%

of the phenotypic variation in this population and has an average additive effect of -14.24 days. These QTLs are most likely the *Ppd-D1* gene described by Beales et al. (2007). Sonora contributed the day length sensitivity allele in the SC population and Foisy contributed the allele in the CF population. This explains why no segregation for the locus was seen in the SF population. The fact that Sonora carries the day length sensitivity allele may seem surprising as it originates from Mexico. However, Sonora is thought to have been selected from a landrace that was brought over to the Americas from Europe with Columbus in 1492 and Shcherban et al. (2015) showed that 91% of spring wheat cultivars in Europe contain the photoperiod sensitive allele *Ppd-D1b*.

A second consistent QTL for days to heading was identified on chromosome arm 5AL in the SF and CF populations. In SF the QTL covered a 4.4 cM region with a peak at 163 cM between markers 5A_10843 and 5A_24477 and explaining 20-21 % of the phenotypic variation. The QTL in the CF population covered a 10.4 cM region with its peak at 92 cM between the markers 5A_1737 and 5A_12135 explaining 18-28 % of the phenotypic variation. Although the markers are not identical they map within a couple of cM of one another in the consensus map of Wang et al. (2014) strongly suggesting that this is indeed the same QTL.

The third QTL for days to heading was on the long arm of chromosome 5B in the SF and CF populations. In SF the QTL covers a region of 2.2 and 10.1 with peaks at 120 and 126 being that there was a slight shift between years from 5B_3483 and 5B_9459 in 2013 to 5B_80245 and 5B_3483 in 2014. This created a larger cM region and a 6 cM shift in the peak of the QTL for 2014. This QTL explains 17-20 % of the phenotypic

variation seen in this population. For CF the QTL covers a 1.6 cM region with its peak around 106 cM between the markers 5B_80245 and 5B_51408 explaining 17 % of the phenotypic variation seen in this population. These populations share the 5B_80245 marker providing validation of the QTL. Additional validation comes from Zanke et al. (2014) who located a gene on chromosome 5B related to the *Hd6* gene family of rice with a major impact on heading time in wheat. They found that the marker Kukri_c10016_369 was the closest linked marker to the locus, and it maps to the same genetic position as 5B_3483 identified in our mapping experiments. This suggests that the QTL identified in two of our populations across multiple years is indeed this same *Hd6* related locus. It is possible that the other QTL identified on 5A and 5D are homoeologous to the 5B QTL. This of course is only speculative and would require further inquiry.

The fourth QTL was identified on the long arm of chromosome 5D in the SC and SF populations. For SC the QTL covers a 2.0 and 24.5 cM region with its peak between 78 and 75 cM respectively. A shift from 5D_17130 and 5D_502 in 2013 to 5D_4695 and 5D_17130 in 2014 cause the differences seen in QTL area and peak position, however, in both years the QTLs share marker 5D_17130. The QTL explains 45-49 % of the phenotypic variation seen in this population. In SF the QTL covers a region of 21.7 cM with a peak at 10 cM between the markers 5D_17310 and 5D_42321 and shares the 5D_17310 in common with the SC QTL. This QTL explains 29-41 % of the phenotypic variation seen in this population.

Finally, the fifth QTL was located on the long arm of chromosome 5D and identified in the SC and CF populations. In the SC population it covers a 19.0 and 10.7

cM region with a peak around 150 and 156 cM, respectively. This QTL explains 40-52 % of the phenotypic variation found in the population. In 2013 the left and right markers were 5D_1682 and 5D_63558 while in 2014 the markers were 5D_63588 and 5D_5776 with marker 5D_63558 appearing in both years. The large region of this QTL is likely due to poor coverage of SNP markers on most of the D genome chromosomes. This QTL was also observed in the CF population where it covered a 7.6 cM region with a peak at 139 cM between markers 5D_63558 and 5D_5776 and explaining 22-25 % of the phenotypic variation in this population. Both populations share markers 5D_63588 and 5D_5776 providing good validation for this QTL.

When comparing days to heading in the greenhouse (2014) and the field (2015) it is apparent that the 18 hours of supplemental light given in the greenhouse greatly reduced the flowering time of the populations. This difference in treatment also enabled us to detect different flowering time loci. In the field only the day length sensitivity and insensitivity loci on 2D were detected, yet when grown under 18 hours of light all other QTLs were able to be identified. These other QTLs could potentially be *Eps* loci given that the photoperiod response was removed via the 18 hours of supplemental lighting provided. However, this speculation would require greater inquiry and further experiments to draw any solid conclusions.

Hybrid necrosis

Several types of hybrid weakness appear in wheat hybrids with regular frequencies. These include hybrid necrosis, hybrid chlorosis, and hybrid dwarfness with

hybrid necrosis being encountered more frequently (Vikas et al. 2013). Hermsen (1963) described hybrid necrosis as a premature and gradual death of foliage in certain hybrids. The trait is controlled by two dominant complementary genes *Ne_1* and *Ne_2* located on chromosome arms 5BL and 2BS respectively (Tsunewaki 1970, Zeven 1972, Nishikawa et al. 1974).

In the three populations tested, hybrid necrosis was rated in the 2013 evaluations only and at which point all lines with unacceptable levels of necrosis were removed. Thus, QTL for hybrid necrosis were identified using the 2013 data for lines with acceptable levels of hybrid necrosis that were genotyped. Two QTLs were identified as being associated with hybrid necrosis in the CF population. The first is on chromosome arm 2BS where it covers a region of 0.8 cM with its peak at 82 cM between markers 2B_31805 and 2B_4614. It explains 22.51 % of the variation seen in the population. The second QTL is on 5BL, covering a 1.6 cM region with a peak at 53 cM between the markers 5B_29636 and 5B_67642. It explains 31.16 % of the variation seen in the population. These two QTL may be the *Ne_1* and *Ne_2* genes but scoring would perhaps have to be repeated to verify the QTLs across years. It is likely that these QTLs were only seen in the CF population since both SF and SC had few lines expressing the trait included in genotyping, whereas CF had more lines expressing the trait that were included in genotyping.

Conclusion

These simple mapping exercises of well-studied traits show that the three populations described here do have a potential in studying important agronomic traits. Given matching of the parental lines and their origins, new traits that may be associated with better stress tolerance in the field can be studied as well. Most importantly these exercises show that the quality and reliability of the genetic maps developed for these populations can be trusted. It has also been demonstrated that the design and relation of these populations allows for cross verification of traits of interest when all three populations are used together in evaluations. This new resource will be available to those who are interested and hopefully they can become a valuable tool for many to have access to.

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Table 1.1: Summary of QTL detected in the (a) SC, (b) SF, and (c) CF populations for traits that were observed during each evaluation in 2013, 2014, and 2015. For AWN the parent contributing the allele for awnlessness is listed. For DTH the parent contributing the allele for longer heading time is listed. For PLTH the parent contributing the allele for greater plant height is listed.

(a)

Trait	Year	Chrom.	Position (cM)[a]	Left Marker	Right Marker	LOD	PVE (%)[b]	ADD[c]	Parent
AWN	2013	3A	94	3A_29898	3A_6929	3.33	4.18	-0.10	Sonora
	2013	6B	103	6B_606	6B_1614	31.20	62.93	-0.39	Sonora
	2014	6B	4	6B_60232	6B_66298	3.17	2.97	-0.08	Sonora
	2014	6B	103	6B_606	6B_1614	41.16	68.30	-0.41	Sonora
	2015	6B	103	6B_606	6B_1614	38.36	72.97	-0.42	Sonora
DTH	2013	5D	78	5D_17310	5D_502	17.60	45.33	-8.65	CBdeM
	2013	5D	150	5D_1682	5D_63558	15.43	40.59	7.99	Sonora
	2014	5D	75	5D_4695	5D_17310	19.79	49.82	-3.39	CBdeM
	2014	5D	156	5D_63558	5D_5776	20.60	52.34	3.38	Sonora
	2014	7D	20	7D_52359	7D_19377	4.35	9.16	-1.41	CBdeM
	2015_Wet	2D	112	2Dx_32130	2Dx_79444	6.39	18.79	7.80	Sonora
	2015_Dry	2D	112	2Dx_32130	2Dx_79444	6.04	18.06	7.57	Sonora
PLTH	2014	4B	93	4B_1339	4B_35605	4.21	9.77	-4.90	CBdeM
	2014	5D	158	5D_63558	5D_5776	11.05	28.66	8.37	Sonora
HNEC	2013	None	N/A	N/A	N/A	N/A	N/A	N/A	

[a]Genetic position rounded to the nearest centiMorgan (cM)
[b]Phenotypic variation explained by the QTL
[c]Estimated additive effect of the QTL

Table 1.1 Continued.

(b)

Trait	Year	Chrom.	Position (cM)[a]	Left Marker	Right Marker	LOD	PVE (%)[b]	ADD[c]	Parent
AWN	2013	5A	266	5A_9620	5A_6716	17.76	37.03	0.26	Foisy
	2013	6B	79	6B_45514	6B_606	12.33	23.76	-0.21	Sonora
	2014	5A	266	5A_9620	5A_6716	20.64	38.86	0.27	Foisy
	2014	6B	79	6B_45514	6B_606	16.58	28.51	-0.23	Sonora
	2015	5A	266	5A_9620	5A_6716	15.53	36.93	0.25	Foisy
	2015	6B	80	6B_68633	6B_84	13.49	29.45	-0.22	Sonora
DTH	2013	5A	163	5A_10843	5A_24477	10.68	21.33	5.02	Sonora
	2013	5B	126	5B_3483	5B_9459	8.97	17.40	4.53	Sonora
	2013	5D	10	5D_17310	5D_42321	13.79	29.05	-5.85	Foisy
	2014	5A	163	5A_10843	5A_24477	10.97	20.59	2.69	Sonora
	2014	5B	120	5B_80245	5B_3483	10.65	20.57	2.72	Sonora
	2014	5D	10	5D_17310	5D_42321	18.73	40.58	-3.81	Foisy
	2014	7D	38	7D_76114	7D_44453	3.56	5.83	-1.44	Foisy
PLTH	2014	1A	33	1A_20387	1A_4741	5.60	14.13	5.79	Sonora
	2014	5D	12	5D_17310	5D_42321	5.91	16.01	-6.20	Foisy
	2014	7A	114	7A_80622	7A_4575	4.39	10.87	-5.10	Foisy
HNEC[*]	2013	2B	142	2B_1188	2B_1631	2.81	9.17	-0.19	Foisy

[a]Genetic position rounded to the nearest centiMorgan (cM)
[b]Phenotypic variation explained by the QTL
[c]Estimated additive effect of the QTL
[*]Not significant based upon the LOD threshold determined by permutation when $\alpha = 0.05$

Table 1.1 Continued.

(c)

Trait	Year	Chrom.	Position (cM)[a]	Left Marker	Right Marker	LOD	PVE (%)[b]	ADD[c]	Parent
AWN	2013	5A	169	5A_9620	5A_6716	31.01	69.44	0.42	Foisy
	2014	5A	169	5A_9620	5A_6716	92.67	96.83	0.49	Foisy
	2015	5A	169	5A_9620	5A_6716	35.88	83.46	0.46	Foisy
DTH	2013	5A	92	5A_1737	5A_12135	11.48	28.71	6.40	CBdeM
	2013	5B	106	5B_80245	5B_51408	7.58	16.96	4.95	CBdeM
	2013	5D	139	5D_63558	5D_5776	10.51	25.68	-6.06	Foisy
	2014	5A	92	5A_1737	5A_12135	7.04	18.17	3.32	CBdeM
	2014	5D	139	5D_63558	5D_5776	8.16	22.38	-3.72	Foisy
	2015_Wet	2D	47	2Dx_7001	2Dx_13208	19.56	64.22	-13.43	Foisy
	2015_Dry	2D	47	2Dx_7001	2Dx_13208	27.35	75.91	-15.05	Foisy
PLTH	2014	5D	136	5D_63558	5D_5776	3.68	7.85	-5.11	Foisy
	2014	6A	84	6A_22320	6A_33567	12.96	33.78	-10.54	Foisy
HNEC	2013	2B	82	2B_31805	2B_4614	10.43	22.51	-0.36	Foisy
	2013	5B	53	5B_29636	5B_67642	13.61	31.16	0.43	CBdeM

[a]Genetic position rounded to the nearest centiMorgan (cM)
[b]Phenotypic variation explained by the QTL
[c]Estimated additive effect of the QTL

Table 1.2: Demonstration of how the three populations can be used to instantly verify QTL within and between populations using awn type QTLs as an example. Verified QTLs are highlighted in green for those coming from Sonora and blue from Foisy.

Population	Year	Chrom.	Position (cM)[a]	Left Marker	Right Marker	LOD	PVE (%)[b]	ADD[c]
SC	2013	3A	94	3A_29898	3A_6929	3.33	4.18	-0.10
	2013	6B	103	6B_606	6B_1614	31.20	62.93	-0.39
	2014	6B	4	6B_60232	6B_66298	3.17	2.97	-0.08
	2014	6B	103	6B_606	6B_1614	41.16	68.30	-0.41
	2015	6B	103	6B_606	6B_1614	38.36	72.97	-0.42
SF	2013	5A	266	5A_9620	5A_6716	17.76	37.03	0.26
	2013	6B	79	6B_45514	6B_606	12.33	23.76	-0.21
	2014	5A	266	5A_9620	5A_6716	20.64	38.86	0.27
	2014	6B	79	6B_45514	6B_606	16.58	28.51	-0.23
	2015	5A	266	5A_9620	5A_6716	15.53	36.93	0.25
	2015	6B	80	6B_68633	6B_84	13.49	29.45	-0.22
CF	2013	5A	169	5A_9620	5A_6716	31.01	69.44	0.42
	2014	5A	169	5A_9620	5A_6716	92.67	96.83	0.49
	2015	5A	169	5A_9620	5A_6716	35.88	83.46	0.46

[a]Genetic position rounded to the nearest centiMorgan (cM)
[b]Phenotypic variation explained by the QTL
[c]Estimated additive effect of the QTL

Figure 1.1: The crossing scheme used for population development. Directionality of the arrows shows from male parent to female parent giving the populations Sonora x CBdeM (abbreviated as SC), Sonora x Foisy (SF), and CBdeM x Foisy (CF).

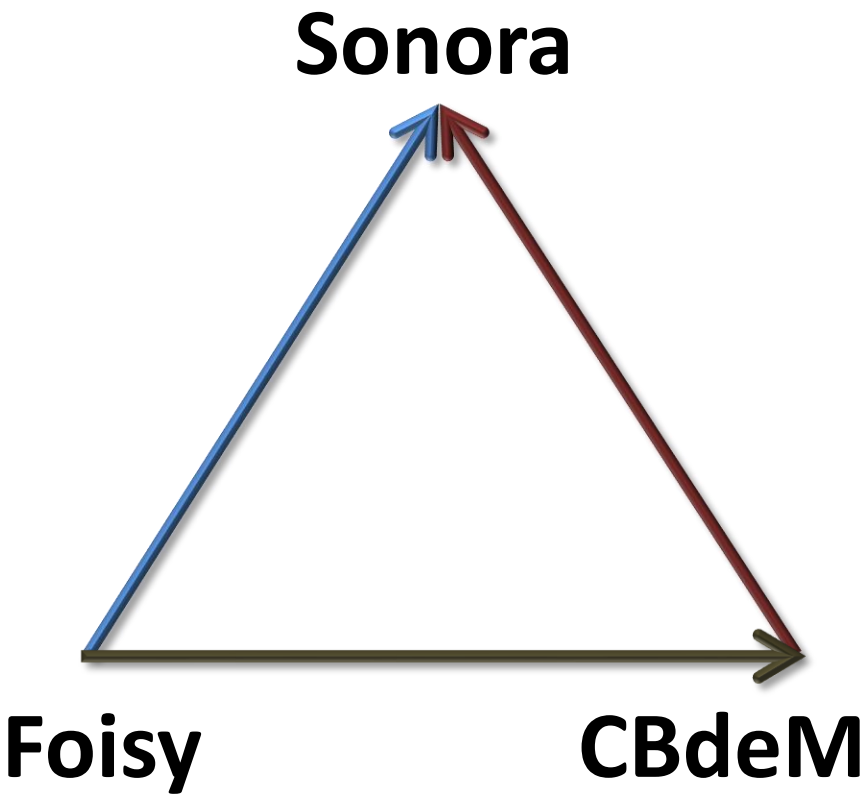


Figure 1.2: Summary of the distribution of traits evaluated in the (a) SC, (b) SF, and (c) CF mapping populations, and the parents in the greenhouse at Riverside, California in 2014. Awns (AWN) were noted as present or absent. The requirement for vernalization (VRN) was determined for plants which did not head before 100 days after planting. Days to heading (DTH) was measured as the number of days after planting when the head emerged from the boot. Plant height (PLTH) was measured from the soil level to the tallest head not including the awns and reported in centimeters.

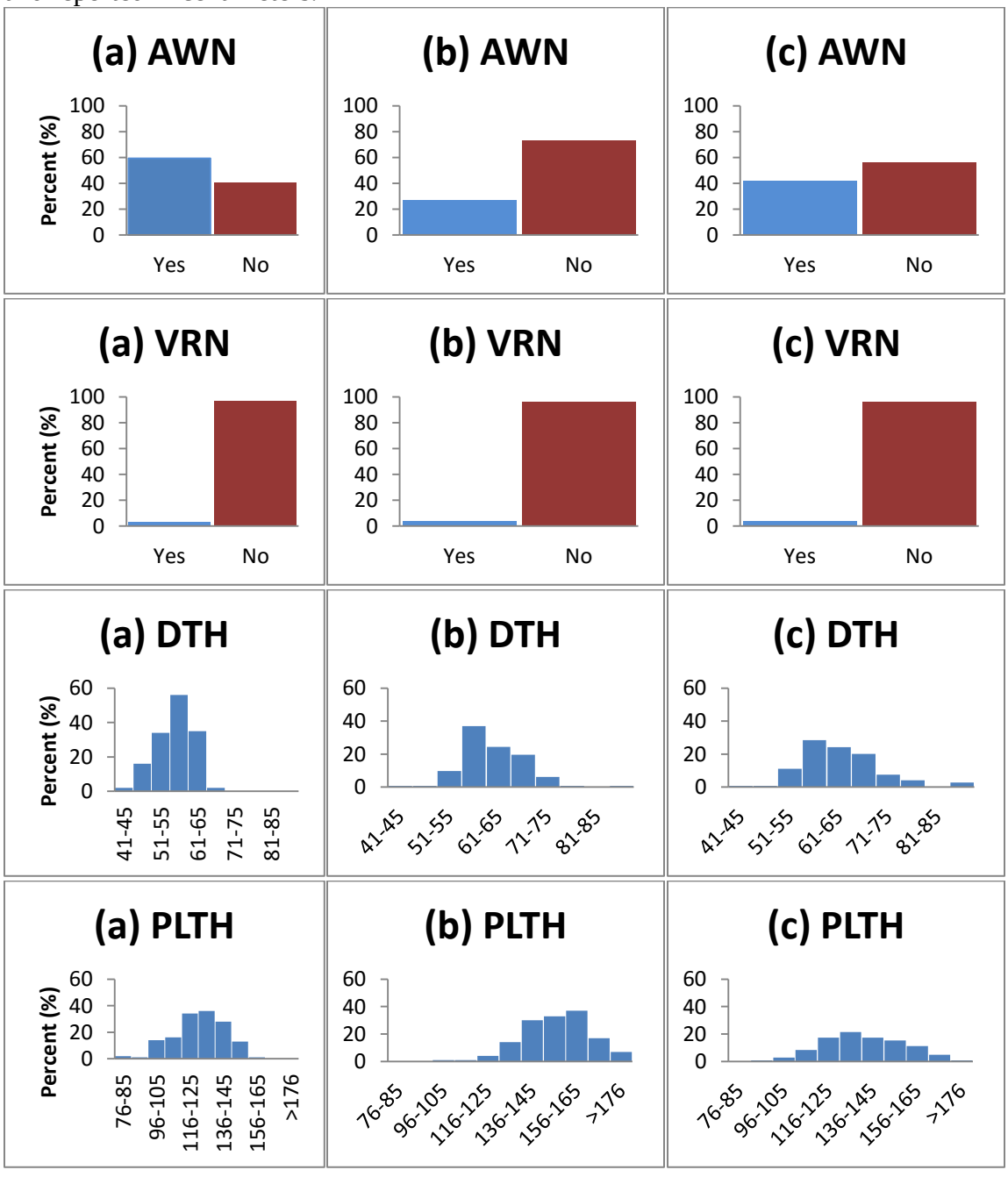
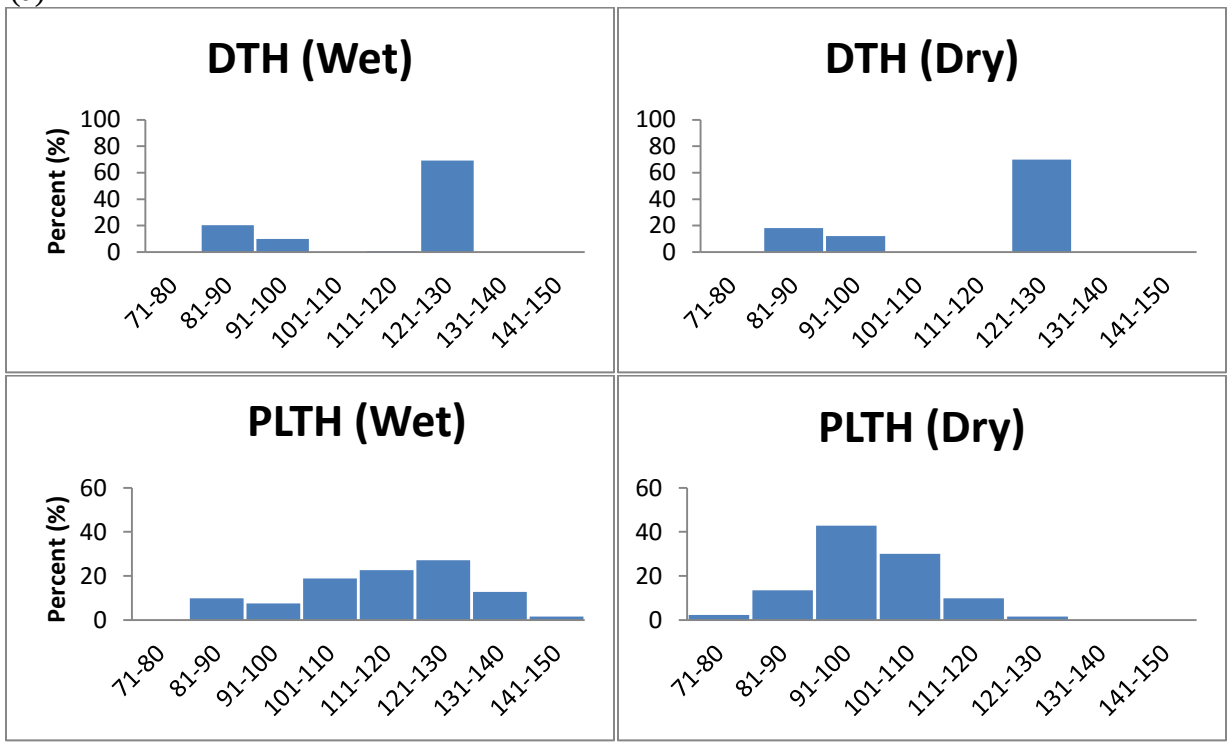


Figure 1.3: Summary of the distribution of traits evaluated in (a) SC, (b) SF, and (c) CF under well irrigated (wet) and limited irrigation (dry) in the field during 2015. Days to heading (DTH) was measured as the time from planting to when the head emerged from the boot. Plant height (PLTH) was measured from the soil level to the tallest head not including the awns and reported in centimeters.

(a)



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(b)

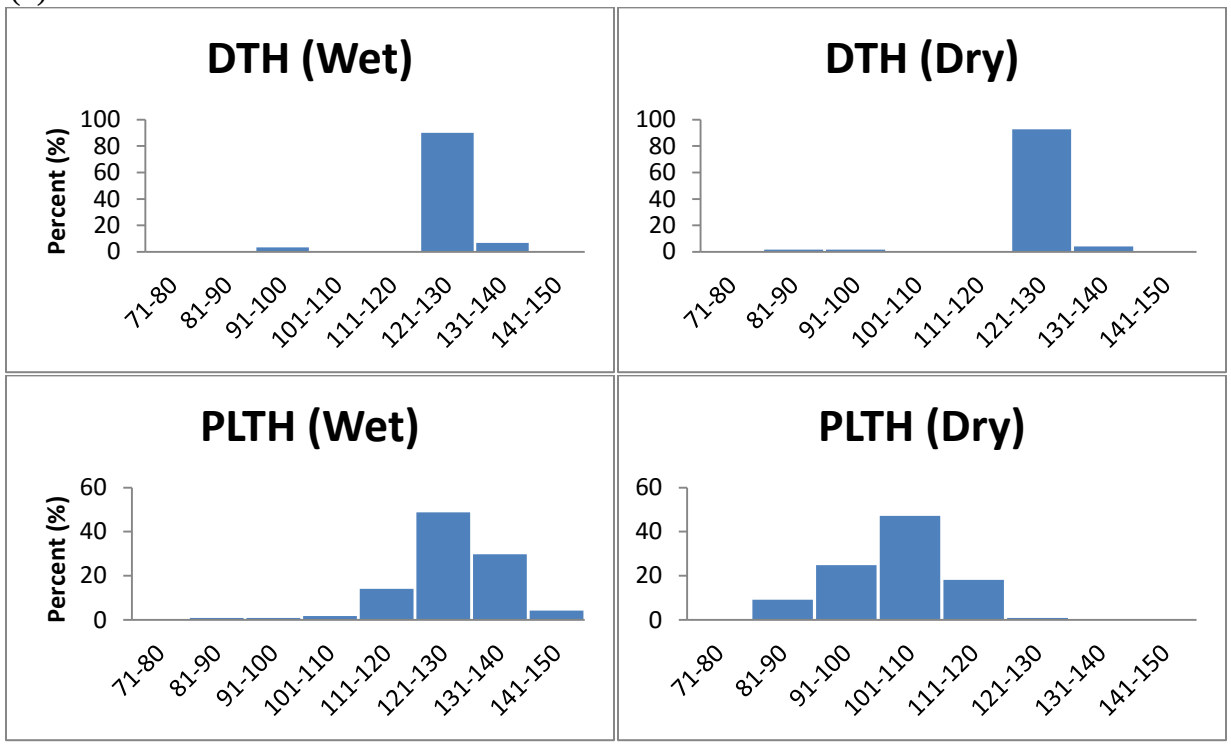


Figure 1.3 Continued.

(c)

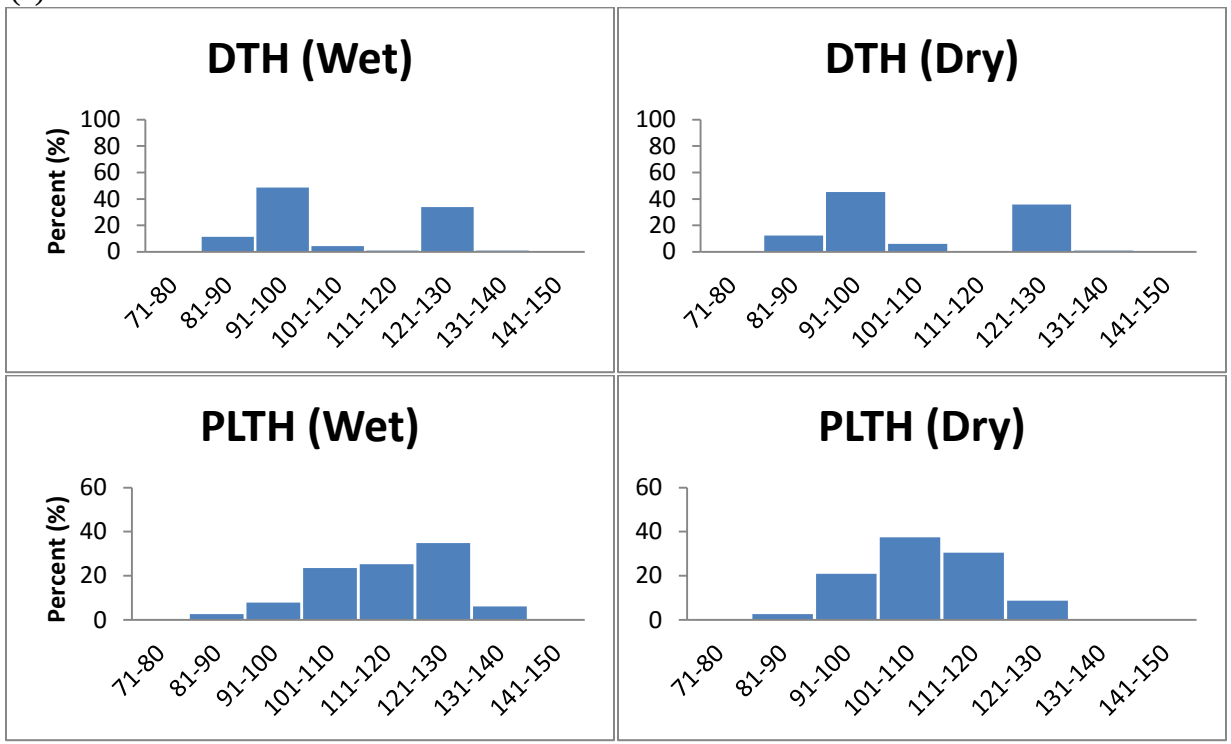
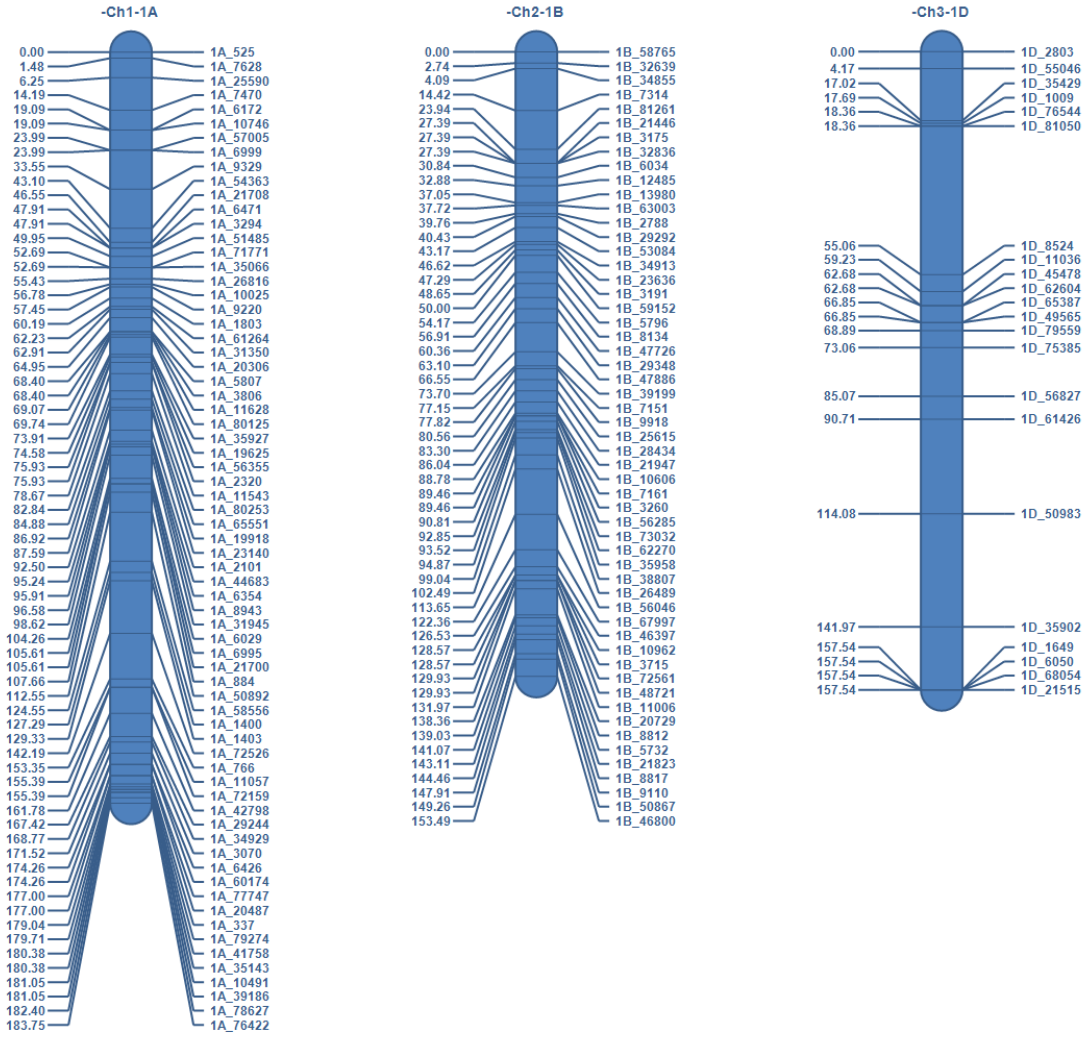


Figure 1.4: Linkage maps for the SC population.



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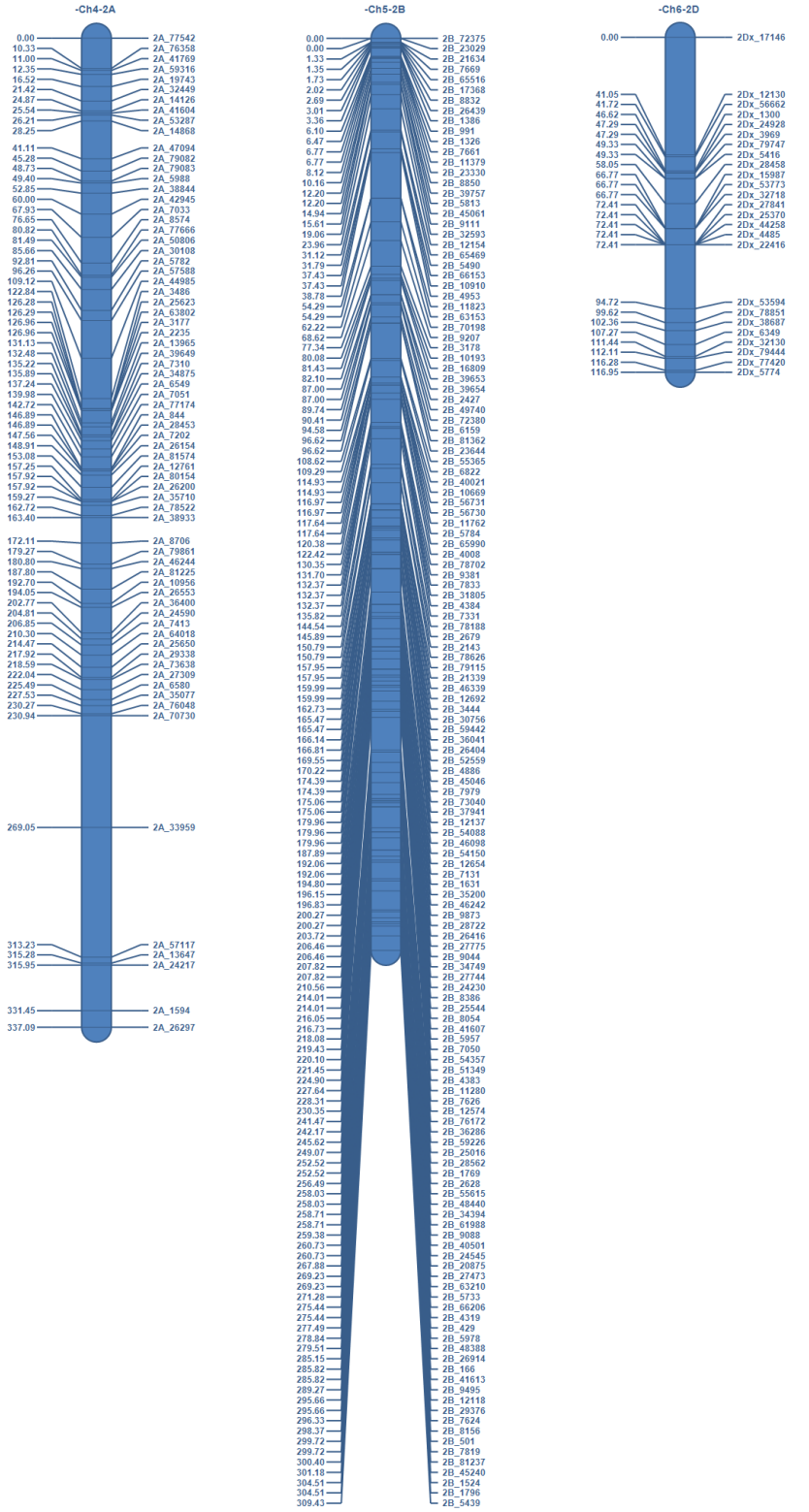

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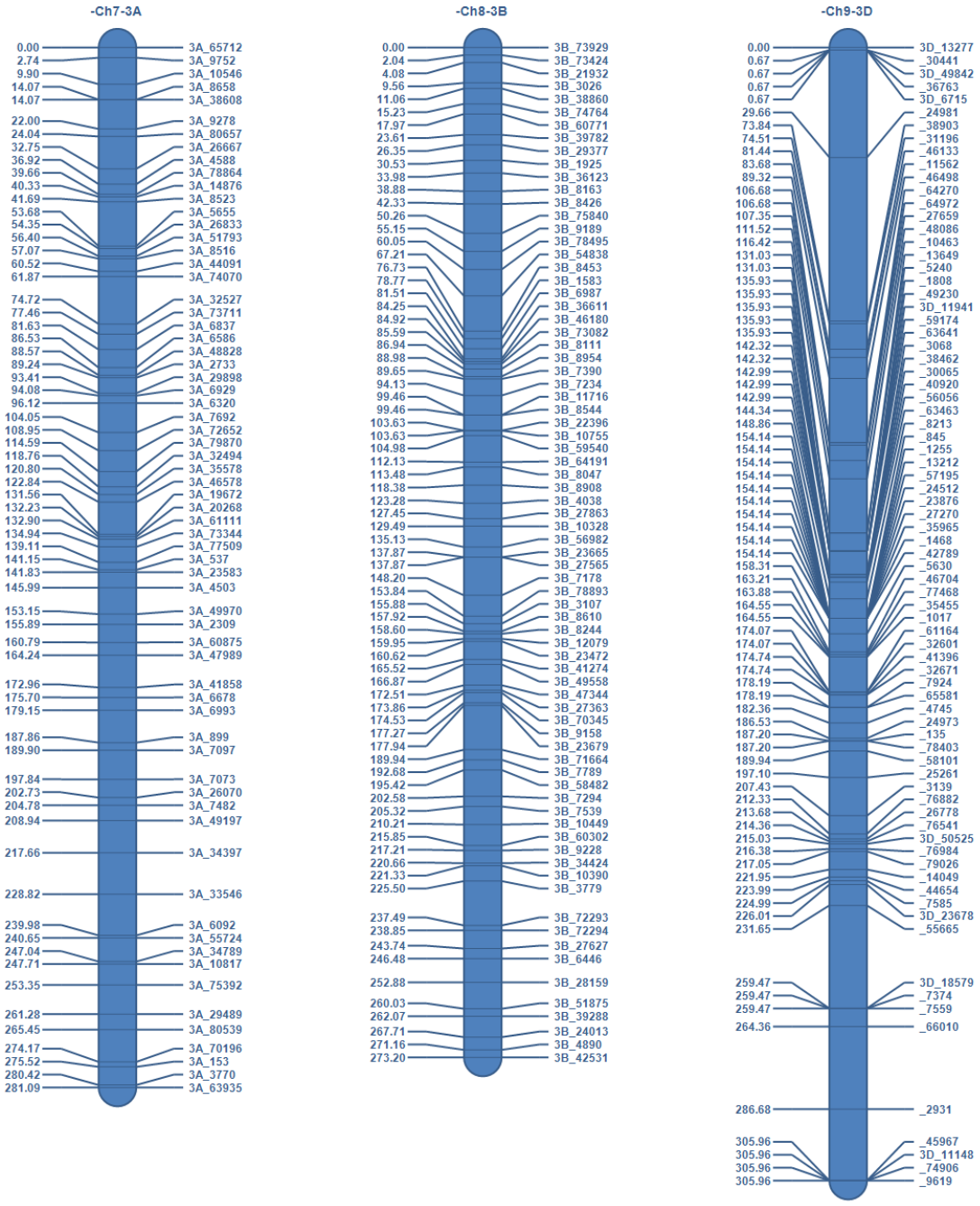

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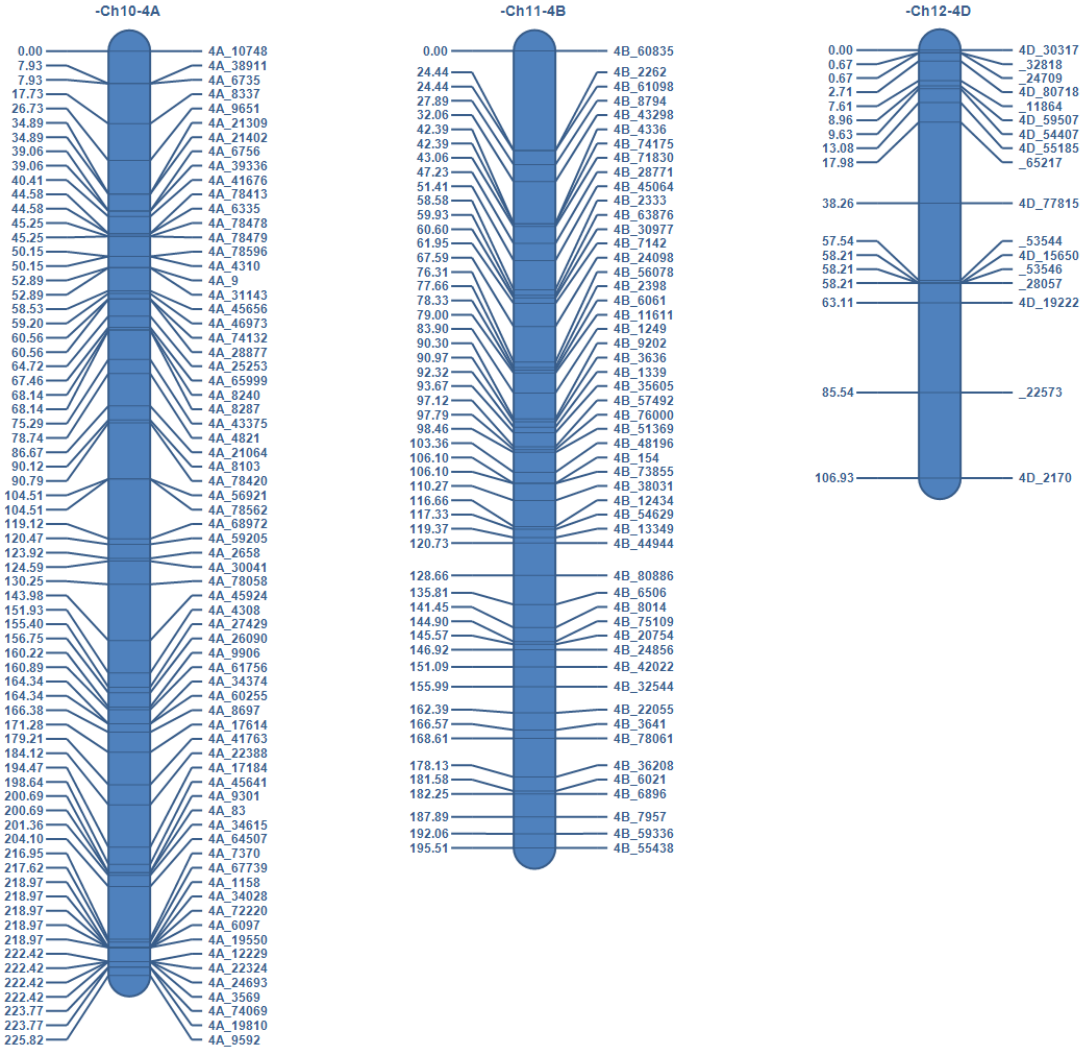

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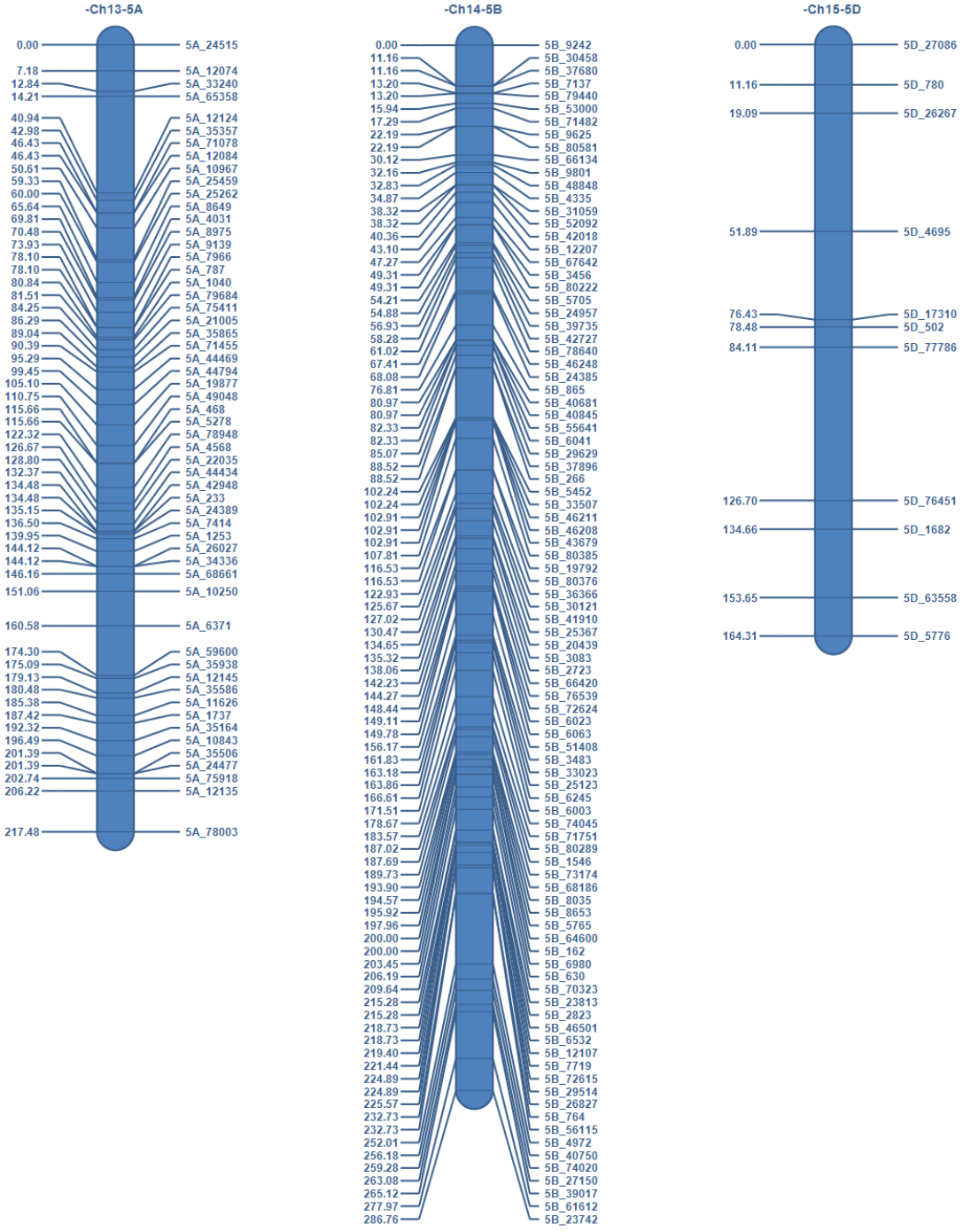

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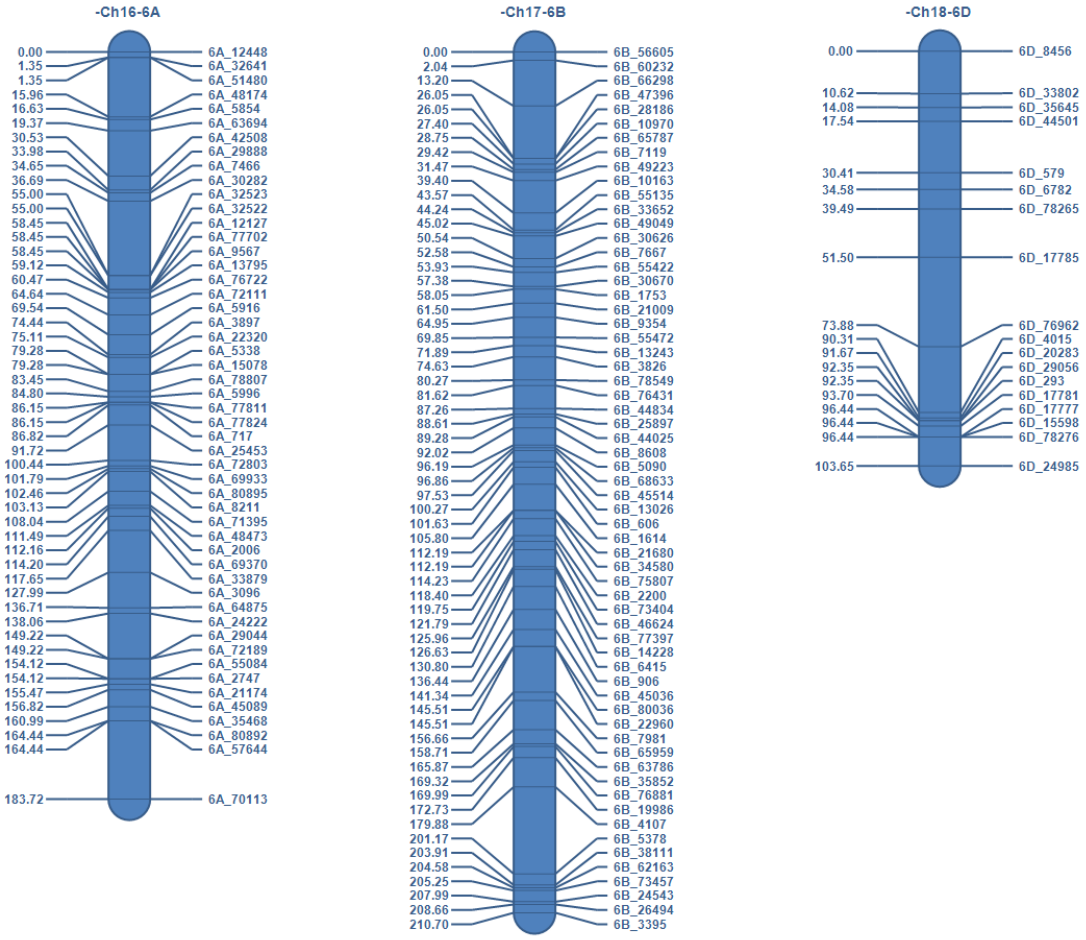

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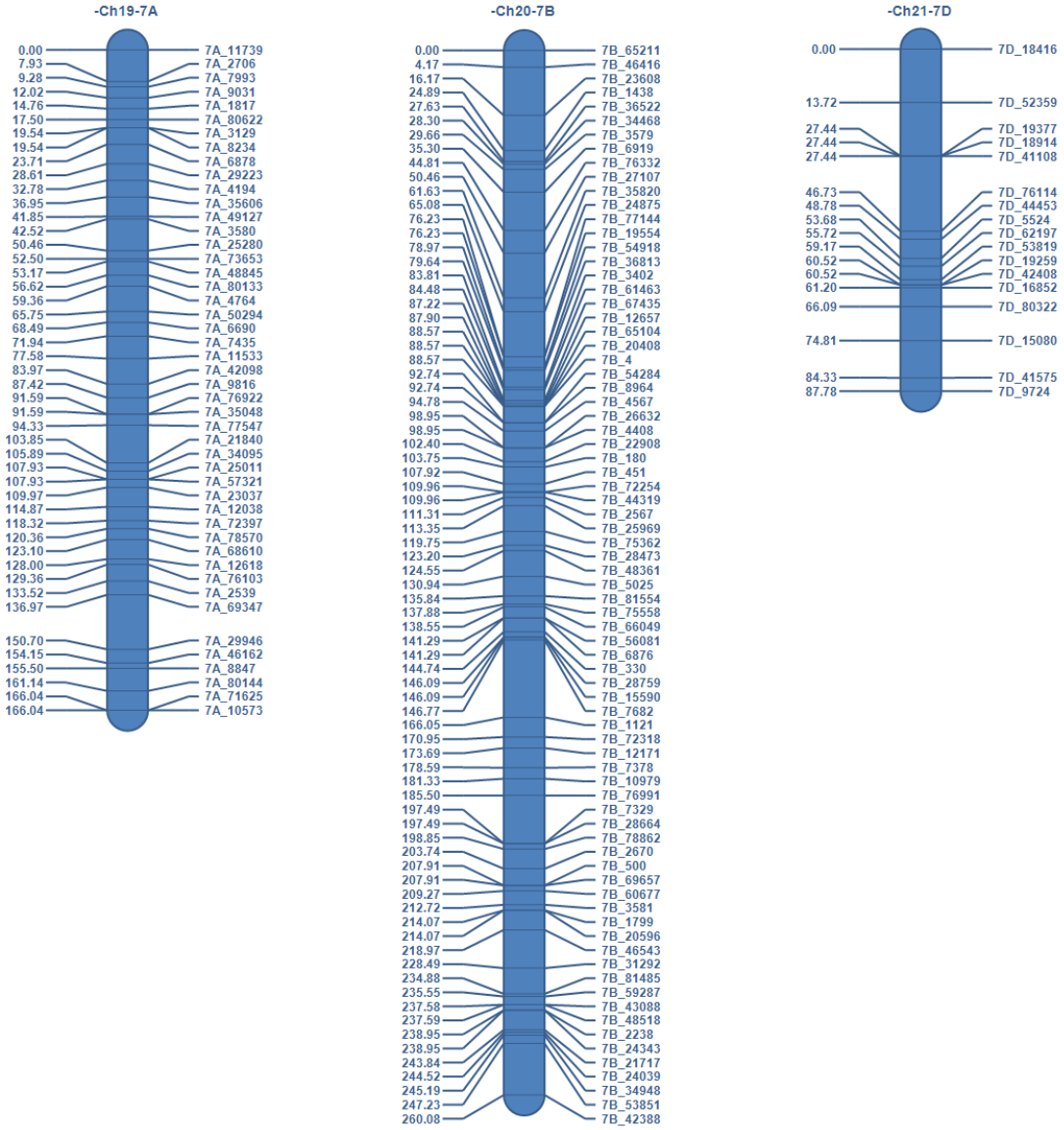
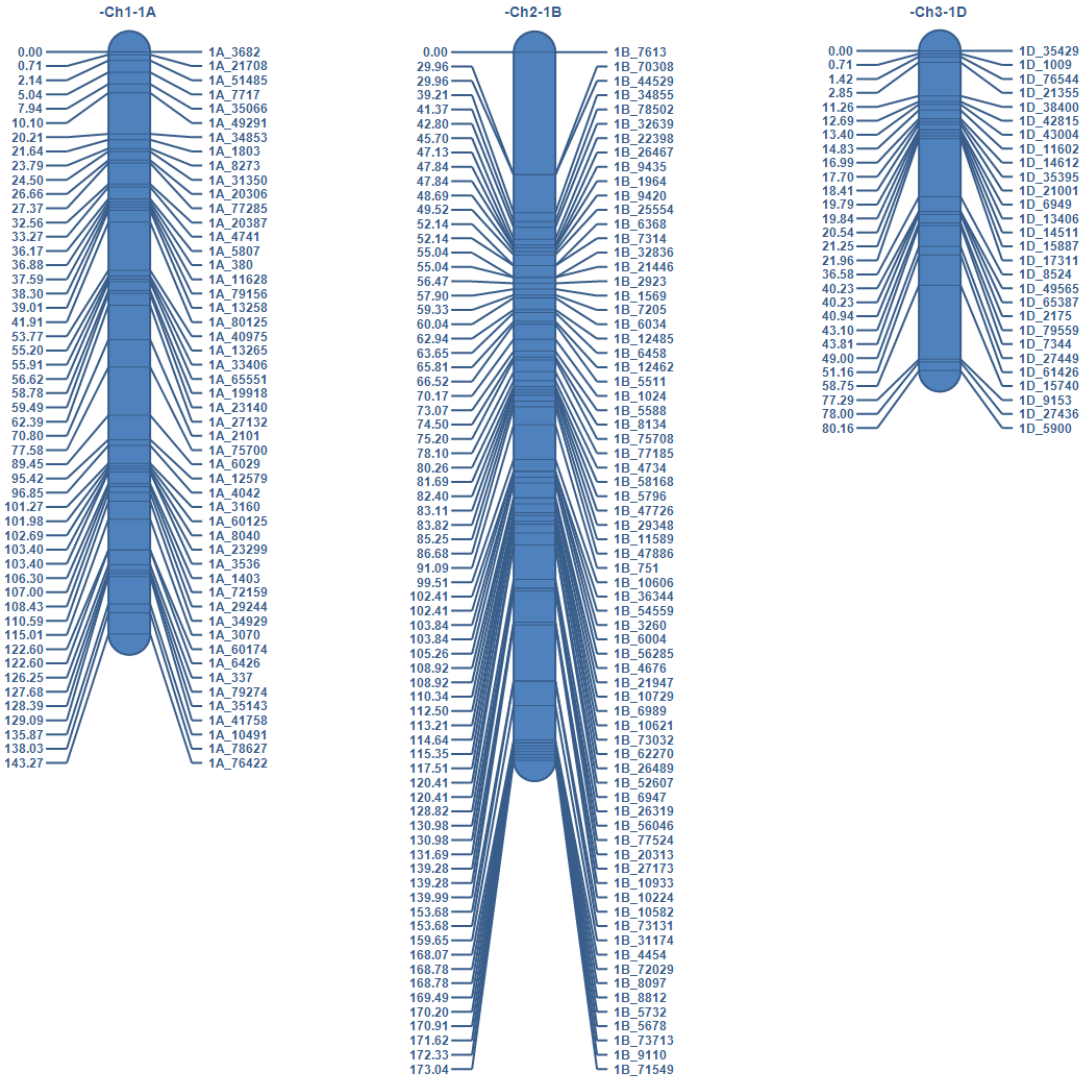


Figure 1.5: Linkage maps for the SF population.



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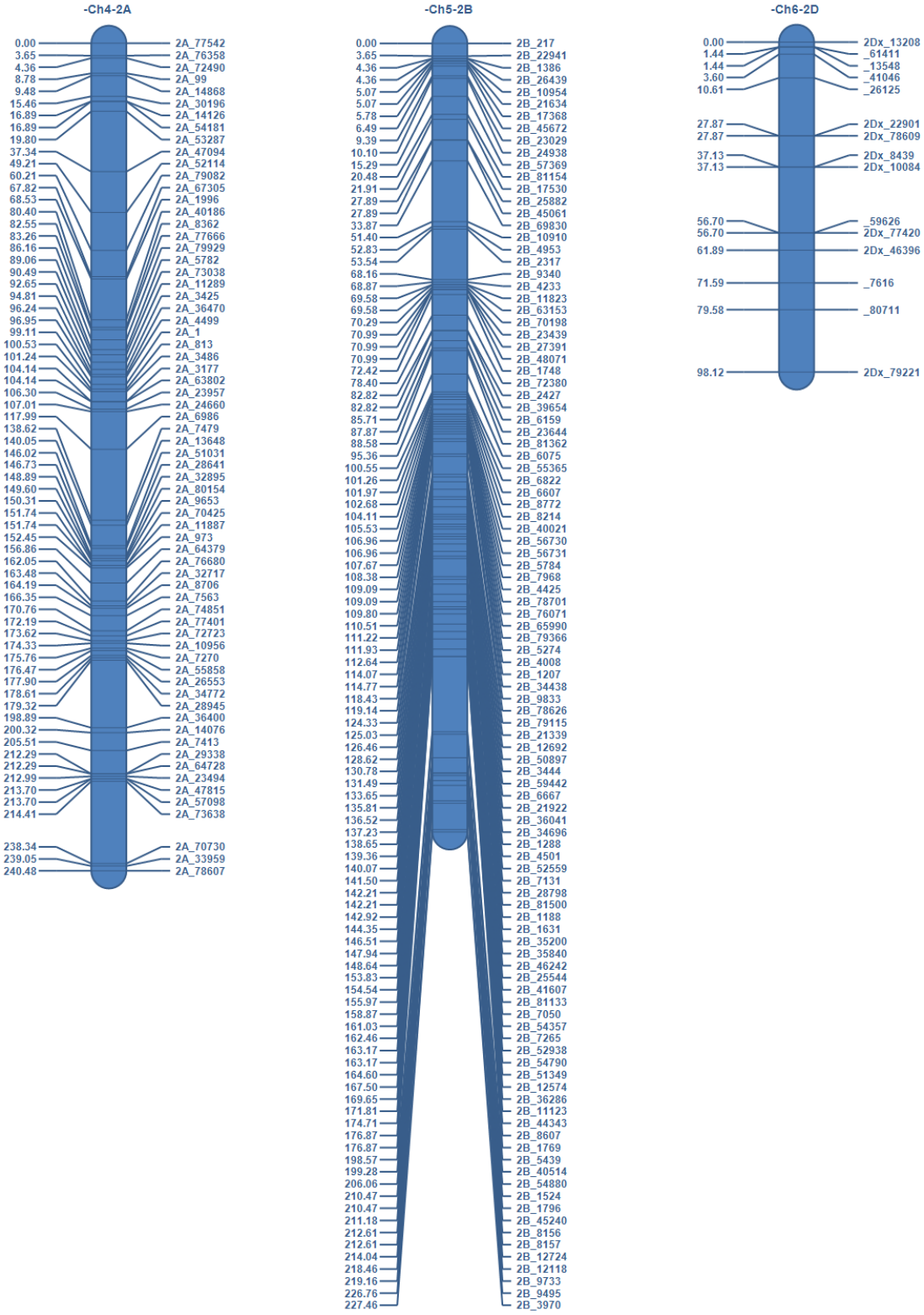

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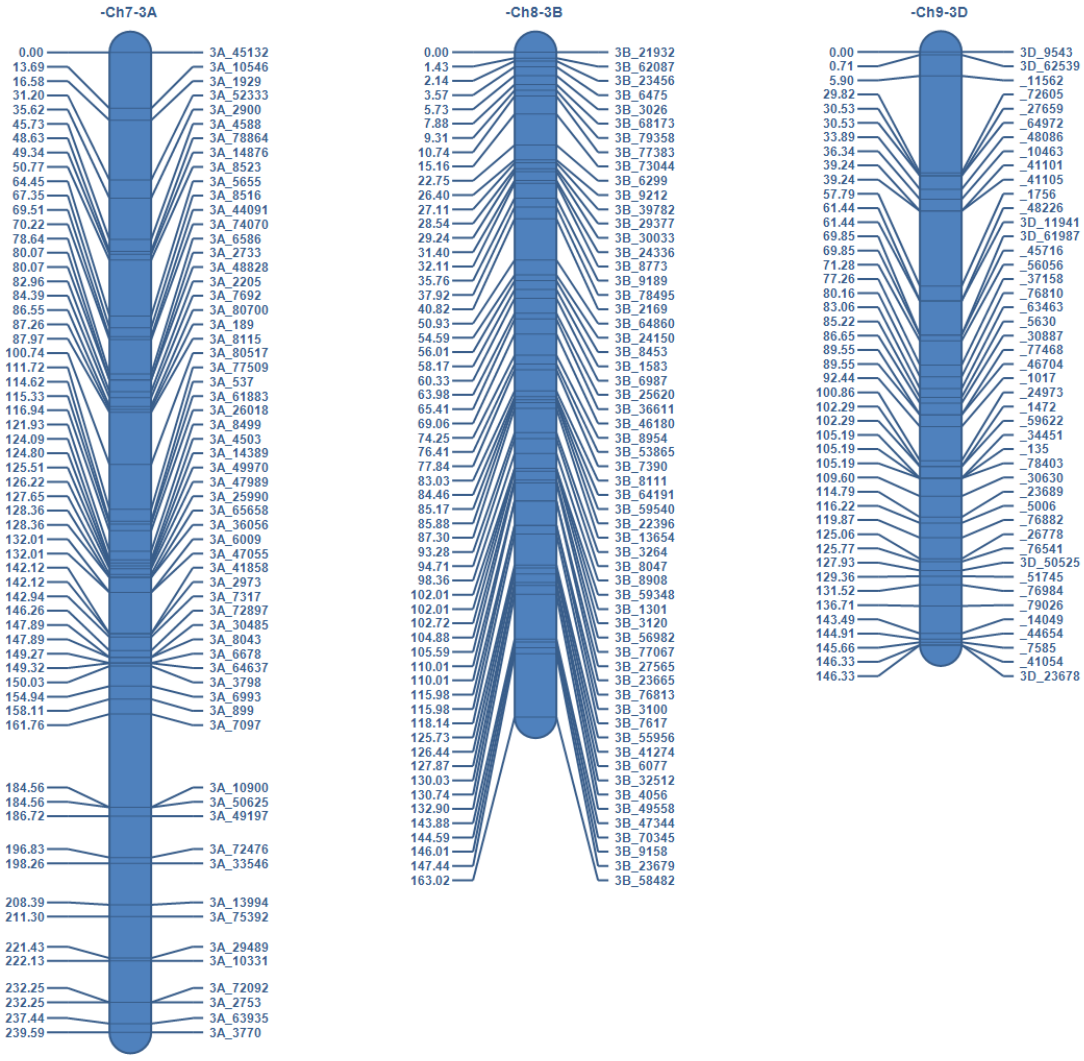

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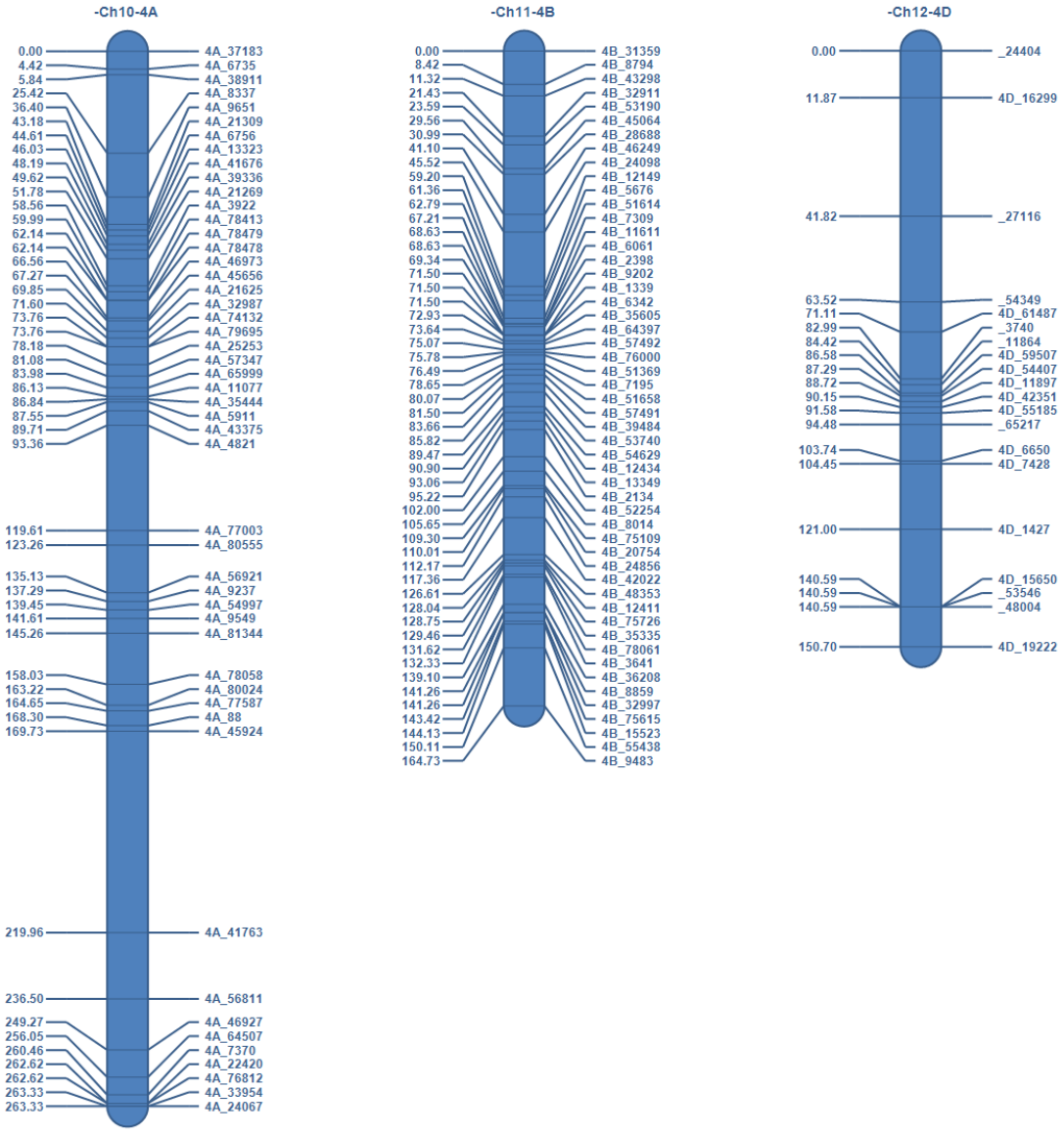

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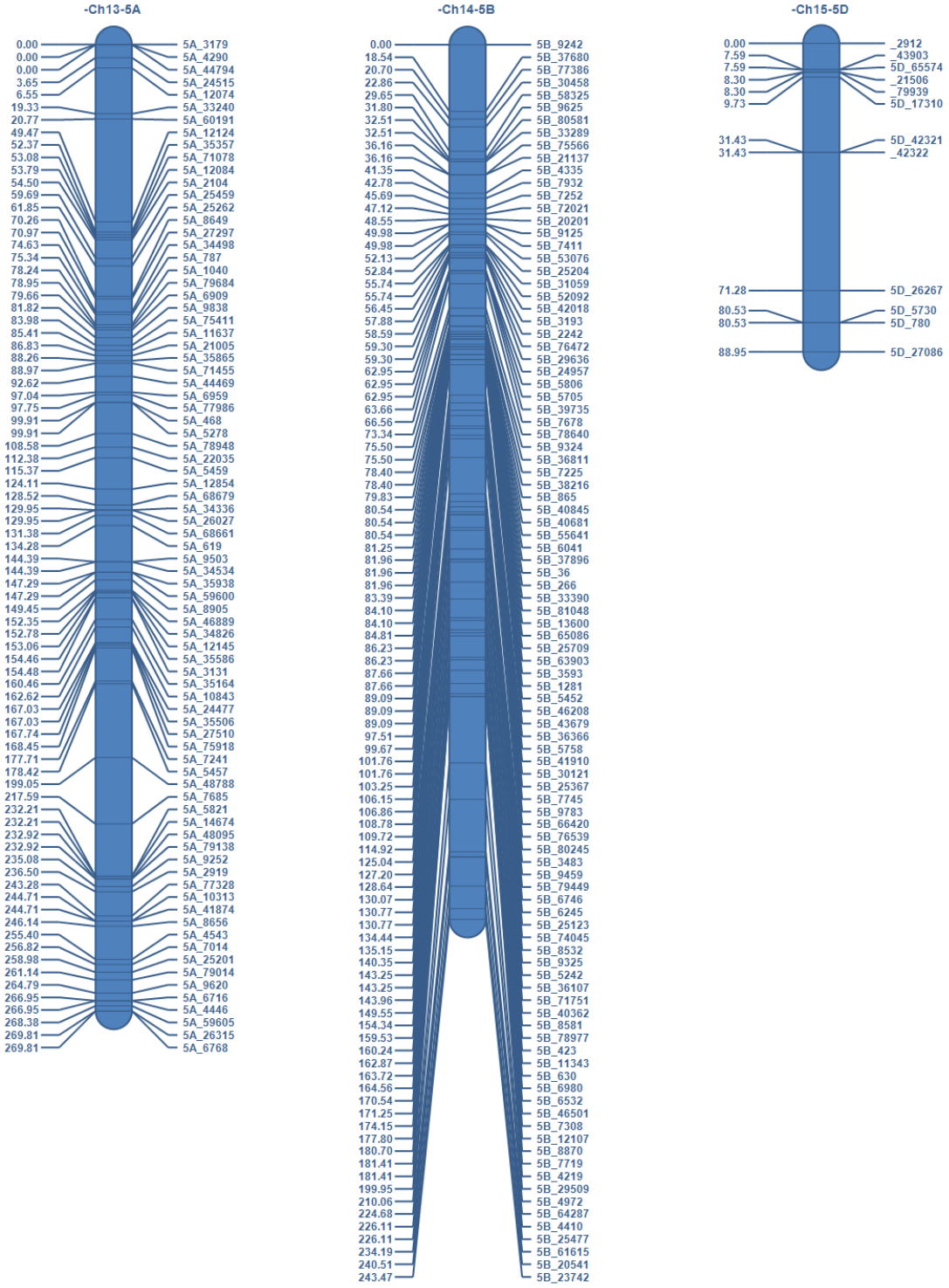

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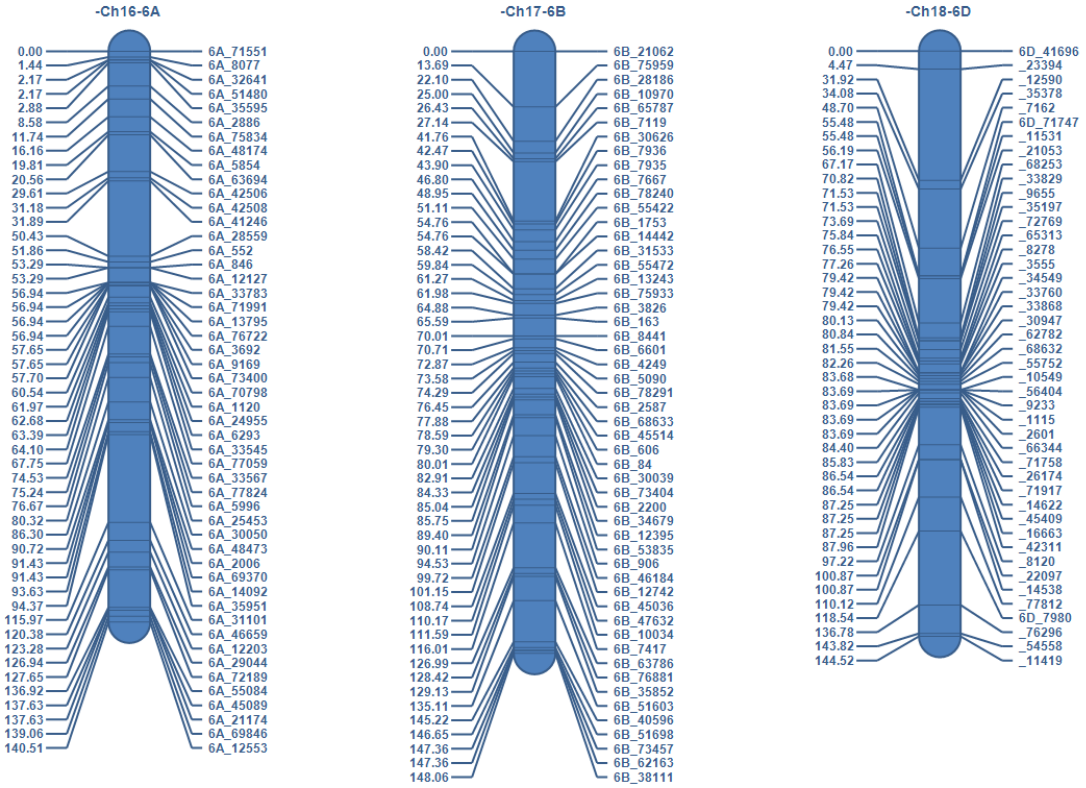

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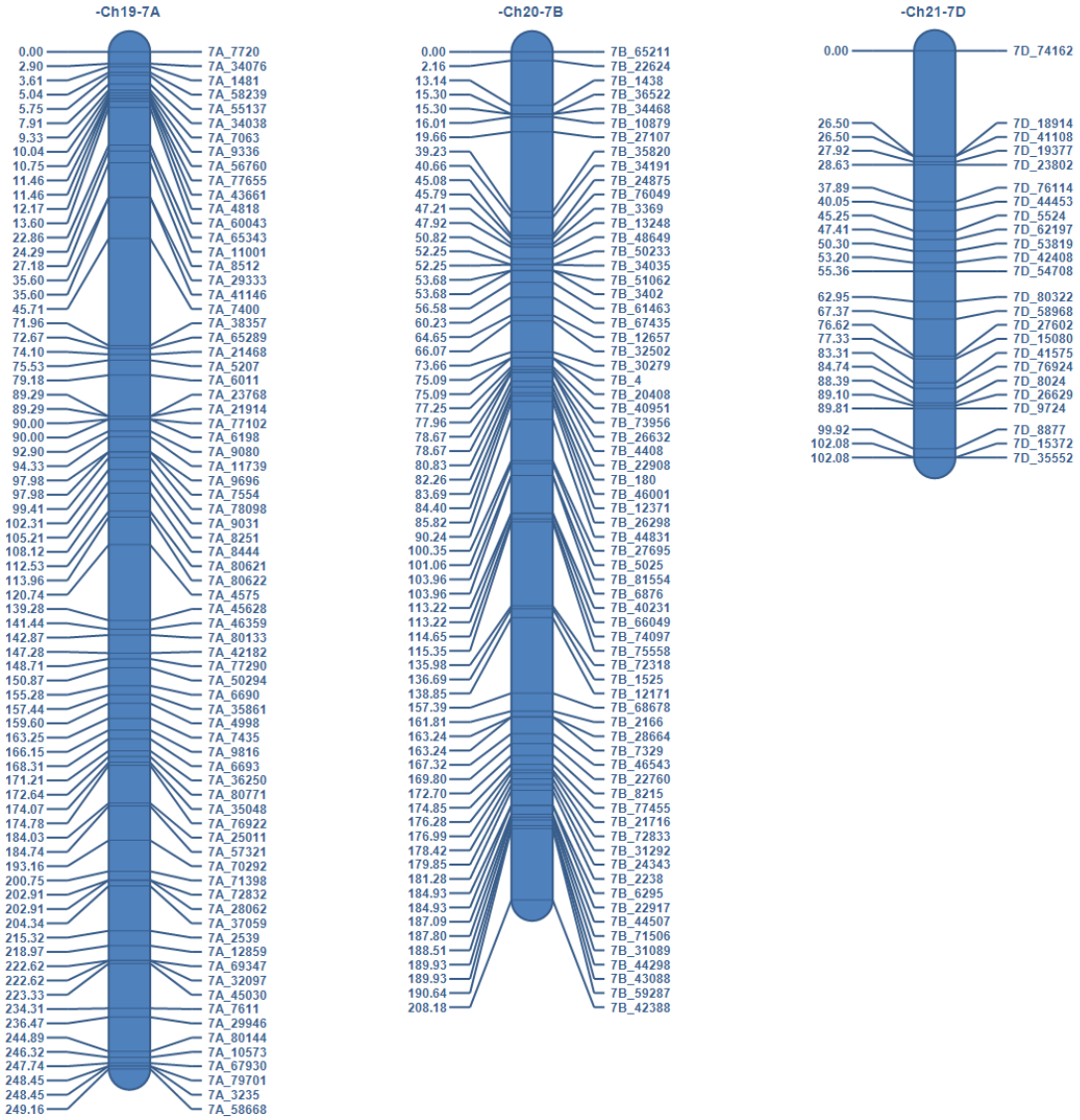
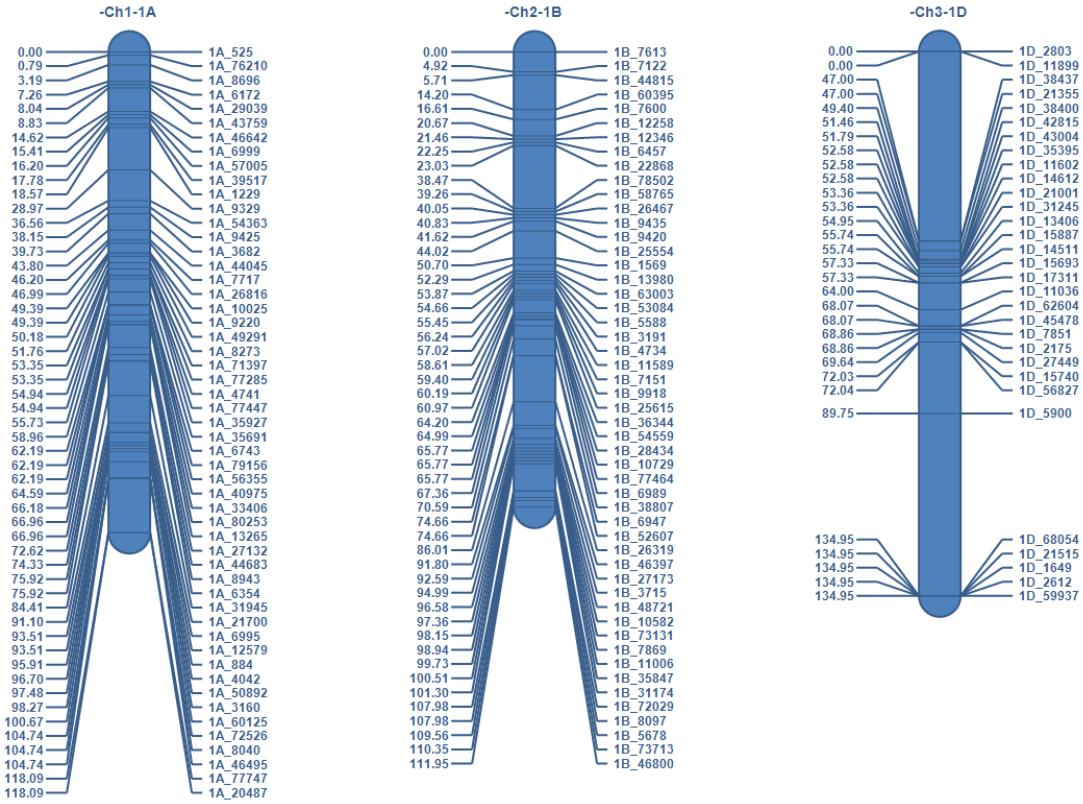


Figure 1.6: Linkage maps for the CF population.



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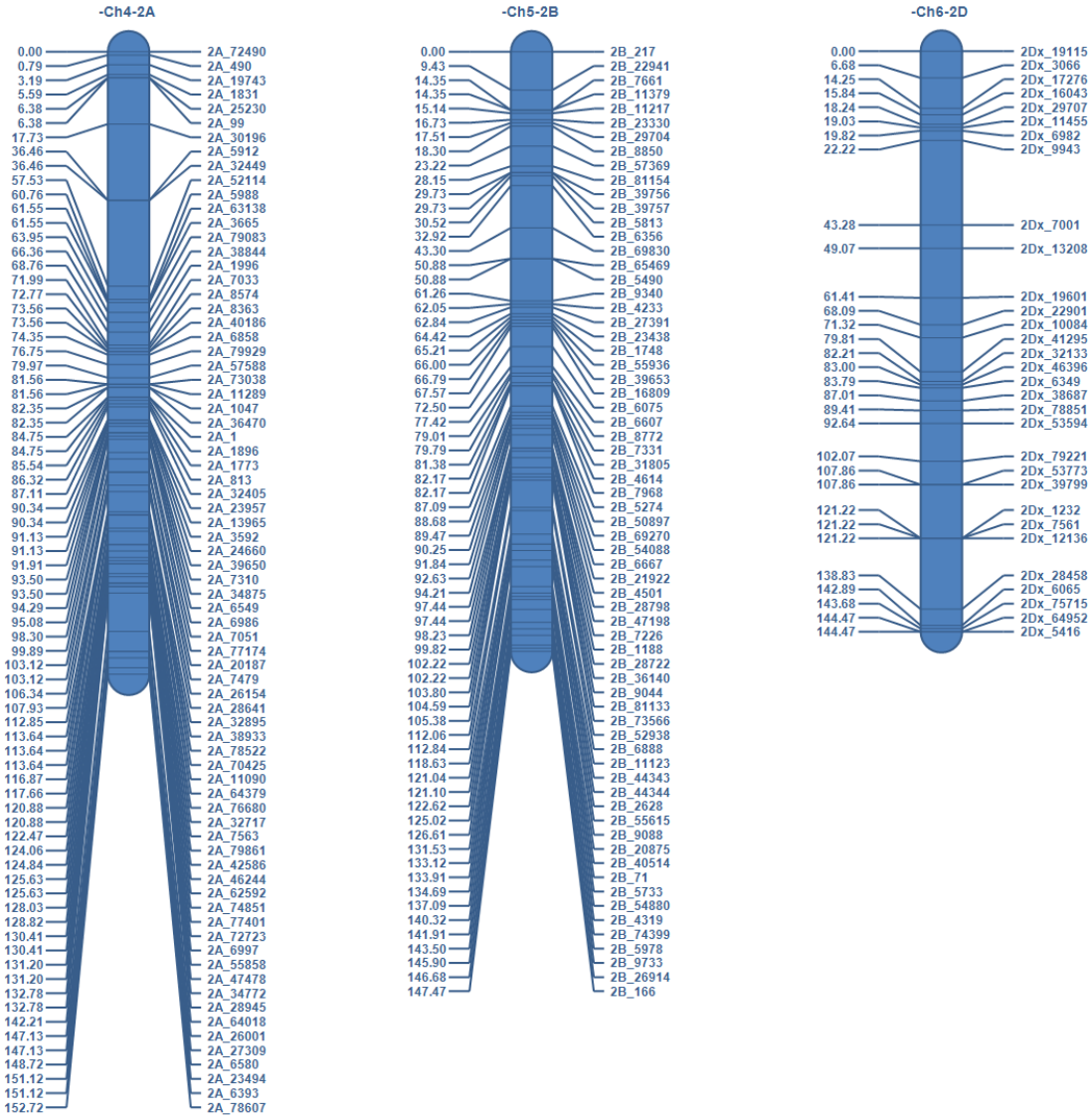

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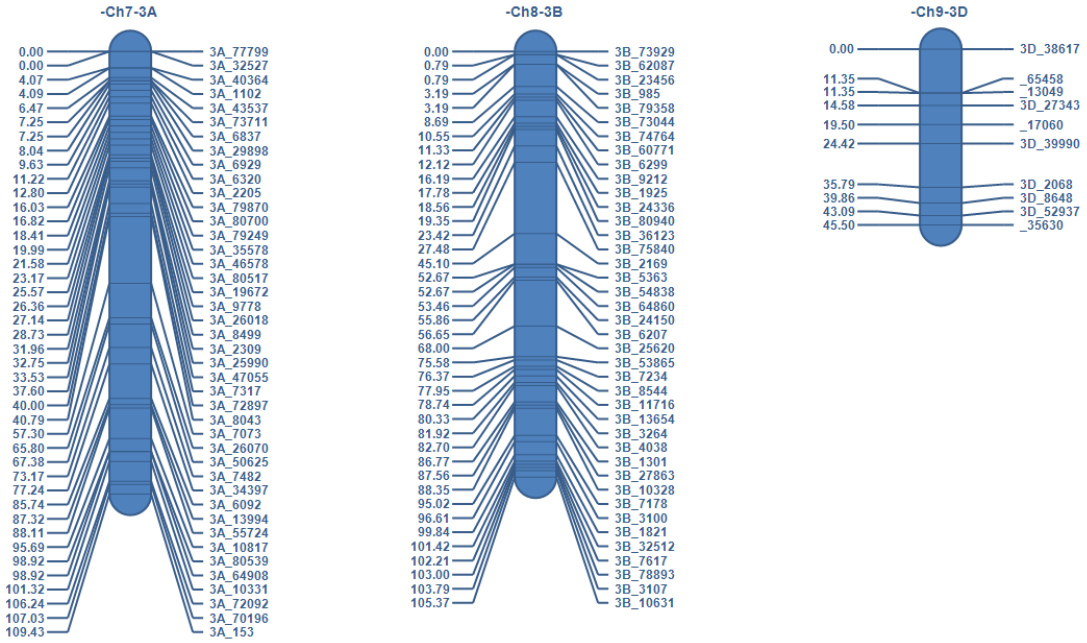

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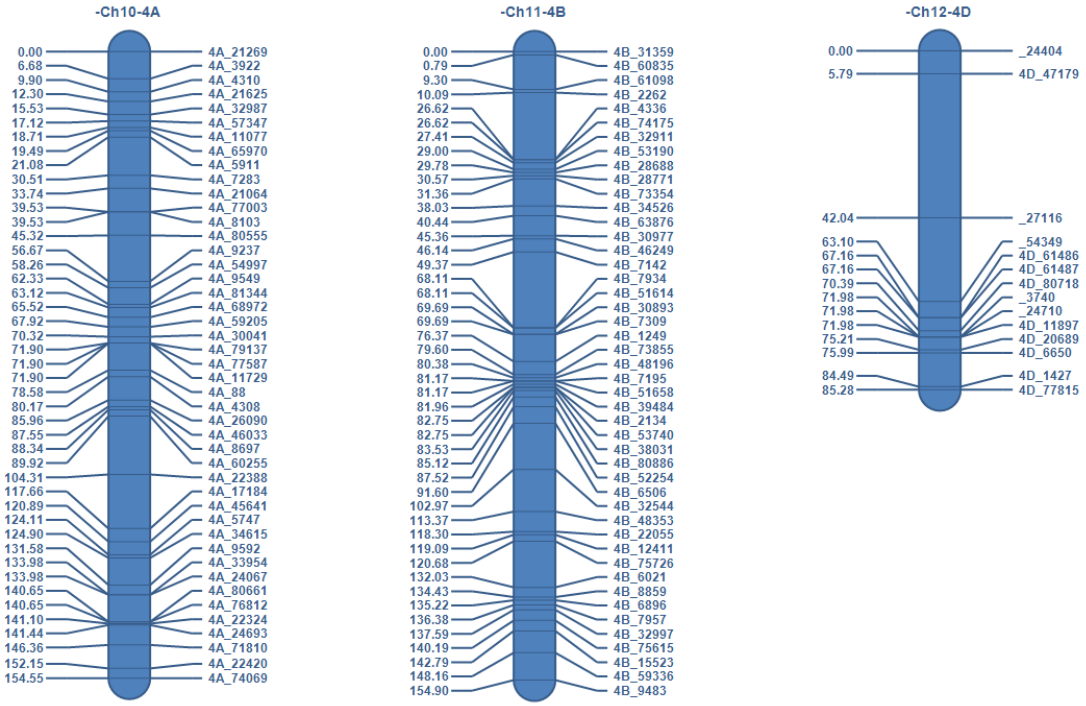

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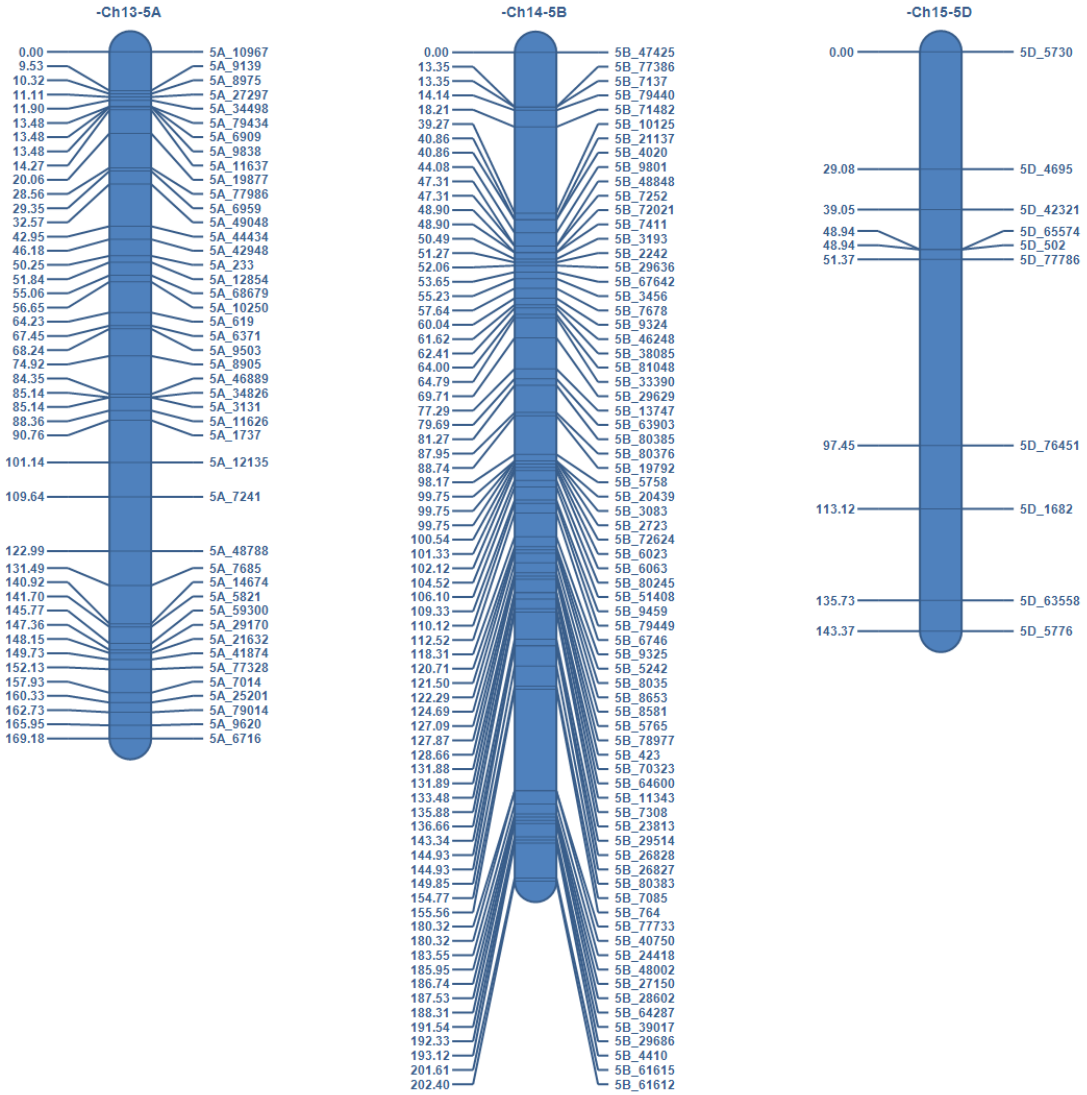

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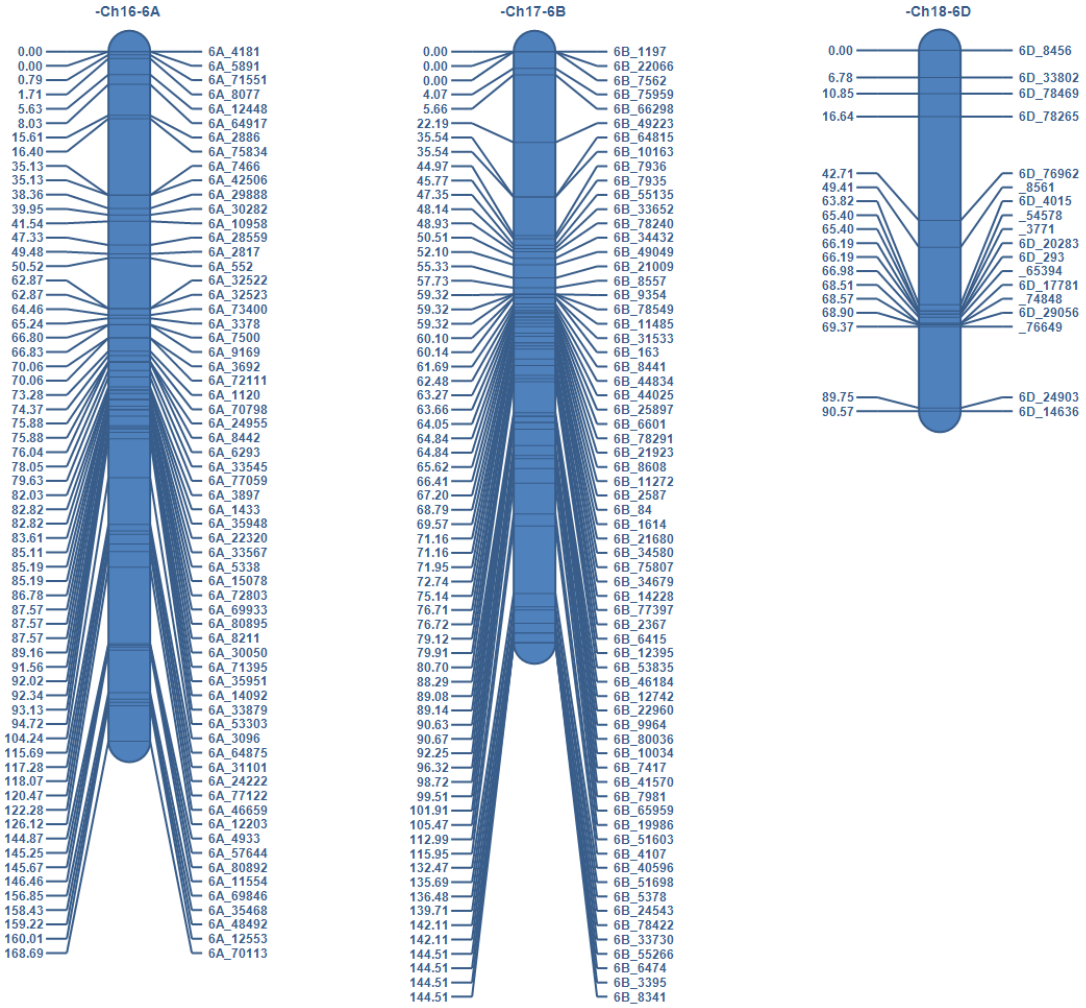

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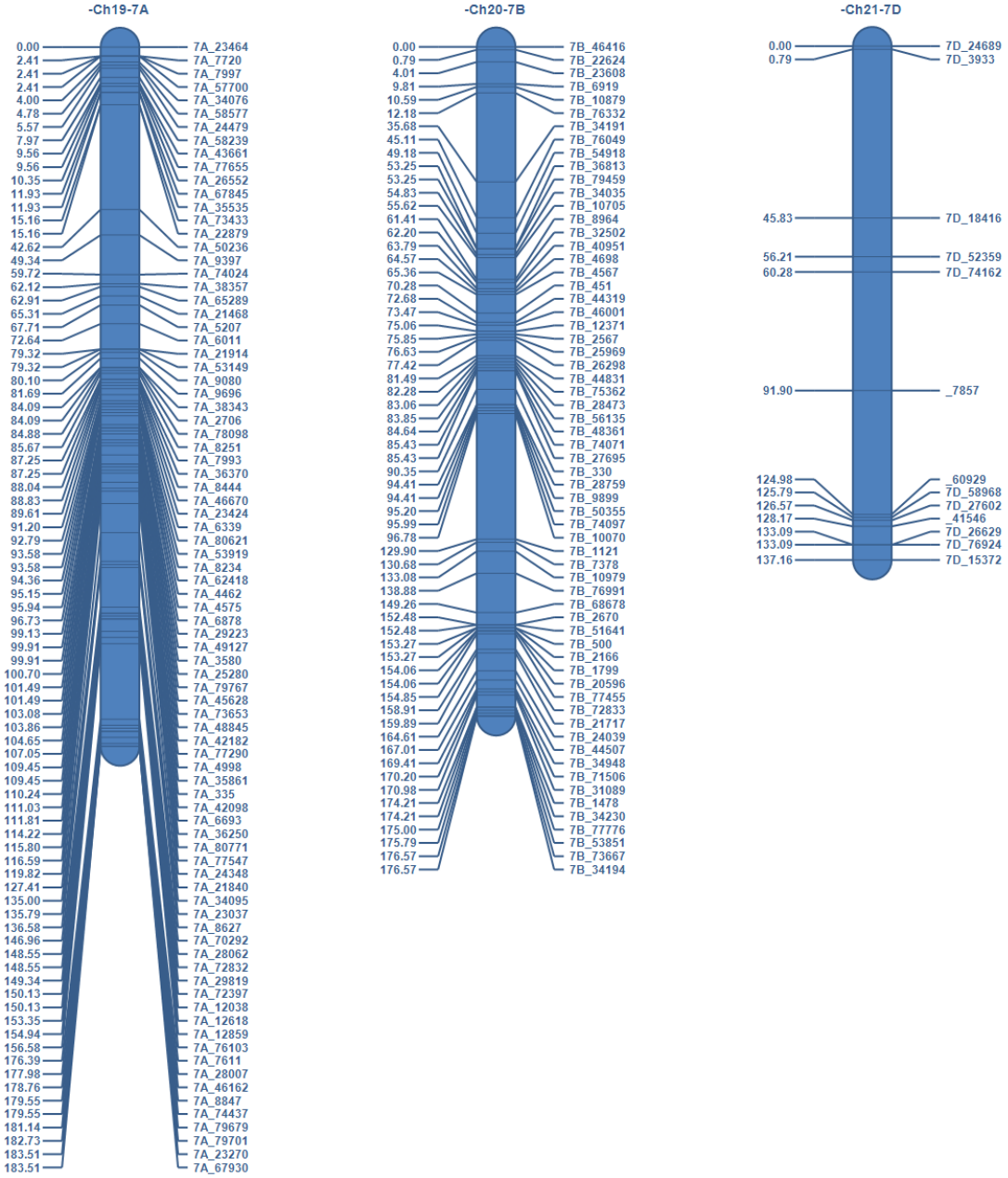

Chapter 2

Genetic Mapping of Quantitative Trait Loci (QTLs) Associated with Seminal Root Angle and Number in Three Populations of Bread Wheat (*Triticum aestivum L.*)

Abstract

Root architecture is related to drought tolerance. Seminal roots are relatively convenient study objects as compared to mature plant root systems and since root system architecture is closely linked to seminal root growth at the seedling stage seminal roots are considered a good proxy. This has led to the idea that selection for root growth angle and number of seminal roots may help to identify genotypes better suited for drought conditions. Here the genetic architecture of seminal root angle and number were investigated using three doubled haploid mapping populations. The crossing scheme of these populations allows for instant verification of quantitative trait loci (QTLs) across populations and genetic backgrounds. All populations showed significant phenotypic variation for both traits and each demonstrated transgressive segregation. In most cases genome regions associated with seminal root angles and numbers were variable from one year to the next and exclusive to a single population. In total 31 genomic regions were associated with both seminal root traits. Considering only the results consistent across both years of experiments, five QTLs for seminal root angle were identified on

chromosomes 2DS, 5BS, 6AL, 7A, and 7BS. Only the QTL on 2DS was verified across two of the three populations; all other QTL appeared only in individual populations. For seminal root number one QTL was identified on 4BL. Correlation analyses for seminal root angle, number, and seed weight revealed interesting relationships to consider for future research. In one population those interactions lead to wrongfully identify QTLs for seed weight as QTLs for seminal root traits. Our findings demonstrate that seminal root angle and number are complex traits and despite high heritability may be more difficult to unwind than previously proposed.

Introduction

With persistent predictions of climate change and increased incidence of drought, crop root systems have gained serious attention. One of the challenges in this line of research is which root traits to focus on and in what environments these traits would be important; another one is to understand how root system traits are associated with one another and what trade-offs at the whole plant level are involved.

Most root morphological traits appear to be regulated by a number of small-effect loci that interact with the environment. This becomes very apparent even at the earliest stages of experiments looking at root biomass and length. Natural plasticity induced by the environment creates large deviations that often obscure the genetic component of the observable phenotype. For these reasons de Dorlodot et al. (2007) suggested that process-based traits such as growth rate, branching frequency and tropism should be studied as opposed to "static traits" such as length, mass, and volume. Some studies have focused on incorporating traits from wild relatives or via new synthetic wheat (Becker et al. 2016, Placido et al. 2013, Reynolds et al. 2007). Others have looked at associations of root system traits and plant height (Bai et al. 2013, Waines and Ehdaie 2007) and many have now begun to focus on seminal root traits.

It has been suggested that, in the context of drought, roots targeting water acquisition deep in the soil profile may be especially important for smaller statured plants such as rice, wheat, and common bean (Comas et al. 2013). By measuring the amount of total water extracted from soil-filled root observation chambers and root growth pattern

data Manschadi et al. (2006) estimated that each additional millimeter of water extracted during grain filling generated an additional 55kg ha^{-1} of grain yield. Lynch (2013) proposed an ideotype for maize roots that included narrow seminal root angles with abundant lateral branching which would optimize water and nitrogen acquisition; this ideotype may also be relevant to other cereal root systems. Narrow seminal root angle generates a root system growing more downward into the soil profile, and presumably, reaching lower soil levels. In contrast, a wide angle of seminal roots appears to promote lateral root growth, a habit that may be beneficial in wetter conditions and under artificial irrigation. With frequent irrigation or rainfall, a root system distributed mainly in the upper soil layers would presumably provide quicker access to water and nutrient, without any cost to the plant for building deep-reaching roots.

Oyanagi (1991) first began to investigate the inheritance of the geotropic response of seminal roots in wheat and concluded that the trait was simple, being controlled by a single locus, and his continued work contributed to the basis for our understanding of seminal root angle physiology in wheat (Nakamoto et al. 1994, 1996; Oyanagi et al. 1993, Oyanagi 1994). Those studies made observations on root distribution patterns and seminal root growth characteristics dependent upon the target environment for which specific cultivars were selected. Typically, cultivars adapted to regions with limited rainfall had narrower seminal root angle and deeper root systems; wheats adapted to environments with higher rainfall and/or irrigation tend to have wide seminal root angles which, presumably, facilitate water and nutrient acquisition from a wider sub-surface area. Following these ideas, Manschadi et al. (2006; 2008) investigated seminal root

angle and discovered a large amount of genetic diversity within the panel of screened cultivars. Their cluster analysis has shown that groups of wheat with similar seminal root characteristics reflected the genetic background and environmental adaptation. Those observations are supported by other research linking root distribution to improved agronomic performance (Cane et al. 2014) and canopy temperature depression under heat and drought stress (Pinto and Reynolds 2015).

Seminal root traits are relatively simple to score and do not require complex experimental systems. This makes them an aspect of choice in root system studies. Drawing ideas from maize studies, Oyanagi (1994) suggested that gravitropic responses of roots would be predictive of wheat root distribution in the soil. That idea was supported by Manschadi et al. (2008) who found that root system architecture is closely linked to the angle of seminal root growth at the seedling stage. Those findings led to a suggestion that selection for the growth angle and the number of seminal roots may identify genotypes better suited for drought conditions.

Measuring root traits of mature plants in the field is a daunting task; for entire mapping populations it is practically impossible. Perhaps for this reason, seminal root traits of seedlings are the favorite research target as they can be measured in several simple experimental set-ups. For all these reasons, studies of seminal root traits appear justified, by providing observations of simple parameters of root architecture, especially when dealing with hundreds of genotypes at a time. At some point all observations of such proxy indicators would have to be verified by screening in the field with a limited number of genotypes. The results presented here add to earlier foundational work, and

begin to unravel the genetics behind some aspects of root system architecture. The emerging picture is far more complicated than originally suggested by Oyanagi (1991). While seminal root angle shows high heritability, it clearly is a quantitative trait with a complicated pattern of inheritance.

Materials and Methods

Plant materials

Seminal root angles and numbers were phenotyped in three doubled haploid populations of bread wheat. These populations were created by pair-wise crossing of three landrace cultivars with contrasting root phenotypes. Cv. Sonora has shallow seminal roots growing at wide angles, and cvs. Foisy and Chiddam Blanc de Mars (abbreviated as CBdeM) have deep seminal roots with narrow angles. Crosses were made in a triangular fashion so that each of the three parents is present in two of the populations. This arrangement provides a built in system for verification of QTL identified across populations and genetic backgrounds. Detailed information about genotyping, linkage mapping and general descriptions of each population can be found in the previous chapter of this dissertation. Populations Sonora x CBdeM (abbreviated as SC), Sonora x Foisy (SF), and CBdeM x Foisy (CF) have 146, 141, and 128 lines respectively.

Growing system

The DH lines were phenotyped using a modified cigar roll method of Zhu et al. (2006). The system is similar to the Cyg germination growth pouches (Mega International, http://www.mega-internaltional.com/index.htm) and the gel based system of Bengough et al. (2004). It consists of two plexi-glass plates 20 cm x 30 cm fitted with spacers, germination paper, racks holding the plates upright and tubs used to hold water.

One hundred seeds were counted and weighed to estimate average seed weight. Seeds of similar size and weight for each genotype were imbibed in water for 24 hr prior to planting. Germination paper wetted with deionized water was placed on one of the two plates, and two seeds of the same genotype were placed with embryos down 5 cm below the top edge of the paper and 8 cm apart. This set up was covered by a second sheet of wet germination paper and a second sheet of plexiglass. The entire set-up was clipped together and placed upright into tubs of water about 8cm deep; this water level was maintained constant throughout the experiment. Seedlings were grown for 7 days at room temperature without supplemental lighting in a head house at the University of California, Riverside between February 2014 to May 2014 and November 2014 to February 2015. Experiments were setup in a randomized complete block design with four replications where replications were treated as blocks. Each replication had two plants from every genotype.

After 7 days plates were removed from the tubs, disassembled, and seminal roots were imaged using a hand held digital scanner (VuPoint Solutions, Magic Wand PDS-ST415-VPS) set to 300 DPI. To acquire images, the top sheet of germination paper was

carefully removed and the top plexi-glass plate was laid over the seedlings so the scanner could be passed over from above. Seminal root angles were measured using the angle tool in ImageJ (http://imagej.nih.gov/ij/) (Schneider et al. 2012). For each plant, the angle between the first pair of seminal roots was measured at approximately 3cm below the embryo of the seed (Richard et al. 2015), as shown in Figure 1.

Statistical analysis and QTL mapping

The analysis of variance (ANOVA) for seminal root angle and number was based on mean values of the experimental units. Broad sense heritability (H^2) was calculated on a mean basis across four replications. Genotype means were used to calculate Pearson's correlation coefficients for seminal root number, seed weight, and seminal root angle.

Genomic regions associated with traits of interest were detected by the software package IciMapping (http://www.isbreeding.net) (Li et al. 2007) using linkage maps for these populations as described previously (Chapter 1) and the mean value of 8 seedlings of each genotype from four replicates. The composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where $\alpha = 0.05$. Markers in the linkage maps were renamed using the index number provided by Wang et al. (2014) preceded by the chromosome designation. QTL consistent between years within populations and/or consistent between populations were considered as verified QTL and named according to McIntosh Catalogue of gene Symbols for Wheat (http://wheat.pw.usda.gov/ggpages/wgc/98/). Following the format of previous publications an uppercase "Q" in the name signifies strong verification of the

QTL and lowercase “q” was used to name QTL that were consistent but warrant further investigation.

Results

Heritability and correlation analysis

Each population showed considerable phenotypic variation for seminal root angle including transgressive segregation. During 2015 one replication of the SC population experienced fungal infection that clearly affected root growth and had to be excluded from all consideration. Table 2.1 (a) shows the means, maximum and minimum, ANOVA results, and broad sense heritability of seminal root angle for each population in both years. There are highly significant differences among genotypes in each population. Coefficients of variance (CV%) were 14.66 and 15.77, 13.87 and 11.55, 16.13 and 16.46 for SC, SF, and CF in 2014 and 2015 respectively. Figure 2.2 shows the frequency distribution for seminal root angle in the three populations based on the combined means of 2014 and 2015 for each genotype. When considering both years, the average seminal root angles for the parental lines were 108.73°, 76.95°, and 63.31° for Sonora, Foisy, and CBdeM respectively. The SC population had a mean of 76.36° ranging from 28.18° to 111.74°. The SF population was similar to SC with a mean of 80.01°, a minimum of 50.2° and maximum of 109.66° degrees. The CF population had a mean of 67.20° degrees with a minimum of 41.29° and maximum of 84.98°. LSD ($p<0.05$) for mean

comparison between genotypes were 14.19°, 14.73°, and 14.16° in 2014 and 21.05°, 13.42°, and 16.31° in 2015 for SC, SF, and CF, respectively.

All populations also showed significant variation for seminal root number. The ANOVA results show that there are highly significant differences between genotypes in each population (Table 2.1b). CV values were 9.69 and 13.62, 11.89 and 10.96, 8.39 and 11.96 for SC, SF, and CF in 2014 and 2015 respectively. Over the two years, the average seminal root numbers for the parental lines were 4.36, 4.38, and 4.49 for SC, SF, and CF. The SC population had a mean of 4.33 ranging from 3.00 to 5.60 seminal roots. SF had a mean of 4.46 with a minimum of 2.82 and a maximum of 5.71 seminal roots. The CF population was very similar to those populations with a mean of 4.57 with a minimum of 2.81 and a maximum of 5.44. LSD ($p<0.05$) for mean comparisons were 0.62 and 0.76, 0.72 and 0.69, 0.55 and 0.73 for SC, SF, and CF in 2014 and 2015 respectively.

Correlation analysis revealed interactions of seed weight with root angle and number, as well as interactions of angle and number (Table 2.2). In the SF and SC populations seminal root number was positively correlated with seed weight ($r = 0.36$ to 0.46). This has been observed before (Robertson et al. 1979, Christopher et al. 2013). In this study, seminal root angle was negatively correlated with seminal root number in two of the three populations: SC ($r = -0.22$ to -0.30) and CF ($r = -0.24$ to -0.33). Additionally, seminal root angle and seed weight were negatively correlated in the CF population ($r = -0.23$ to -0.35).

Heritability values for both traits were fairly high. For seminal root angle they were 70.18 and 68.41, 64.88 and 60.53, 52.30 and 56.55% for SC, SF, and CF in 2014

and 2015, respectively. For seminal root number the heritability values were 63.10 and 63.92, 46.96 and 49.96, 57.05 and 53.53 % for SC, SF, and CF in 2014 and 2015 respectively.

QTL discovery

In most cases associations of root system characteristics with specific genome region varied between populations and within populations from one year to the next. Over two years of the experiment and with all three populations taken together, a total of 31 genomic regions showed statistically significant associations with the seminal root angle and number (Table 2.3). Seminal root angle was associated with 12 chromosome regions in the SC population, located on chromosomes 2D, 3B, 4A, 5A, 6A, 6B, 6D, and 7B. In the SF population, five regions on 2D, 5B, 6B, and 7B were identified and another five regions were identified in the CF population, on chromosomes 5B, 6A, and 7A. The chromosome region with the single largest effect for the seminal root angle was located on chromosome 2D in the SC population. Its estimated effect was equivalent to 7.33° of the total root angle, and it was responsible for 21.42% of the population variation. The region with the lowest, but statistically significant effect for root angle was identified in the CF population, accounting for an estimated 2.90° of the root angle and explaining 9.40% of the variation observed in this population.

For the seminal root number, nine genomic regions were identified in the three populations. Of these, four were identified in the SC population, on chromosomes 4A, 5B and 7A. The SF population had only one region, on chromosome 4B. The remaining

three regions were identified in the CF population on chromosomes 1B, 6B, and 7D. The region with the largest effect was on chromosome 4A in the SC population, with an estimated effect of -0.25 roots per seedling explaining 17.32% of the total variation. The region with the lowest but statistically significant effect was identified on chromosome 6B in CF, with an estimated effect of 0.15 roots per seedling, explaining 8.41% of the population's variation.

For the purpose of this study, only those genome regions that showed consistent associations with specific traits within a given population over both years were considered as verified QTLs (Table 2.4). In the SC population three such regions were identified, located on chromosomes 2D, 6A, and 7B (Figures 2.3, 2.4, 2.5). The region on chromosome 2D was 4.17 cM region with a peak at 113 cM between markers 2Dx_79444 and 2Dx_77420 in 2014. It accounted for 25.99% of the phenotypic variation seen in the population that year. In 2015, the region was located between markers 2Dx_32130 and 2Dx_79444 covering a 0.67 cM with a peak at 112 cM. That year it explained 21.42% of the phenotypic variation seen. The allele for wider seminal root angle was contributed by Sonora.

The second QTL was located on chromosome 6A. In 2014 it was between markers 6A_72189 and 6A_55084 covering a 4.90 cM region with a peak at 151 cM. It explained 7.04% of the phenotypic variation that year. In 2015, this QTL formed a peak at 155 cM between markers 6A_55084 and 6A_21174, it coved 1.35 cM and explained 7.21% of the variation for the trait. The allele for wider seminal root angle was contributed by Sonora.

The third QTL is located on chromosome 7B. In both years it was located between markers 7B_3402 and 7B_61463, covering 0.67 cM with a peak at 84 cM . This QTL accounted for 7.32 and 7.21 % of the phenotypic variation in the population in 2014 and 2015 respectively. The allele for wider seminal root angle was contributed by Sonora.

In the SF population only one genome region, located on chromosome 2D, was consistent through both years (Figure 2.6). In 2014 it was located between markers 2Dx_10084 and 2Dx_77420 covering a 19.57 cM region with a peak at ca. 52 cM and explaining 22.96 % of variation. In 2015, it was between markers 2Dx_78609 and 2Dx_10084 covering a region of 9.26 cM with its peak around 36 cM and explaining 13.24 % of the observed phenotypic variation. The allele for wider seminal root angle was contributed by Sonora.

In the CF population, two genome regions consistently associated with seminal root angle, located on chromosomes 5B and 7A (Figures 2.7, 2.8). The QTL on chromosome 5B in 2014 covered a 2.4 cM region with a peak around 58 cM between the markers 5B_7678 and 5B_9324 explaining 9.40% of the variation seen in the population. In 2015 its peak appeared at the 50 cM position on the map, covering a 1.59 cM region between the markers 5B_7411 and 5B_3193 and explaining 19.50 % of the phenotypic variation. The second QTL was located on chromosome 7A. In 2014, it appeared between 7A_9696 and 7A_38343 covering a 2.40 cM region with its peak at 84 cM, explaining 9.98 % of the variation; in 2015 it covered a 2.40 cM region with its peak at 99 cM between markers 7A_6878 and 7A_29223, explaining 3.37 % of the phenotypic variation

seen in the population that year. The allele for narrow root angle on chromosome 5B was contributed by Foisy and CBdeM contributed the allele for wide seminal root angle on 7A.

Seminal root number was associated with fewer genomic regions and only one such region was verified across both years, on chromosome 4B in the SF population (Figure 2.9). In both years *QRN.ucr-4B* was located between markers 4B_12434 and 4B_13349 spanning 2.16 cM with its peak around 91cM. In 2014 it explained 15.81% of the phenotypic variation and in 2015 it explained 12.77% of the variation seen in the population. This region was contributed by Foisy. In the CF population a QTL on chromosome 1B was highly suggestive of a QTL in 2014 and significant in 2015 (Table 3), however, that QTL will remain unverified yet deserves further inquiry in future studies.

Discussion

Phenotypic variation for seminal root angle and number

Each of the three tested population showed large phenotypic variation for both seminal root traits measured in this study. The largest range in seminal root angle was between Sonora and CBdeM with average seminal root angles of 108.73° and 63.31° respectively (Figure 2.2). The least difference, but still statistically significant, was between CBdeM and Foisy which have more similar seminal root angles of 63.31° and

76.95° respectively. The distribution patterns among progenies imply considerable trait complexity.

Seminal root number also showed significant differences among progeny in each population (Table 2.1b). All three parents typically had five seminal roots with few variations between replication giving averages of 4.36, 4.38, and 4.49 seminal roots for SC, SF, and CF respectively. The occurrence of less than five seminal roots is likely explained by environmental interaction and associations with seed weight. Since all parents typically develop five seminal roots it is not surprising that the three populations have similar means and ranges. As will be discussed later, the lack of consistent QTLs for seminal root number may suggest that this trait is heavily influenced by the environment and seed weight. However, one consistent QTL was identified which also suggests that there is a genetic component as well.

Additionally, heritability values were relatively high for both traits in all populations but it does not seem to promise any ease of selection for breeding efforts. As will be discussed it certainly doesn't hint at simplicity for the genetics of these traits.

QTL analysis

The 90K SNP array was used on eight mapping populations of doubled haploids to order SNPs along individual chromosomes and 44,345 of those were mapped to one or more of 46,977 loci (Wang et al. 2014). Due to differences in polymorphism among different sets of parents, only a fraction of all mapped markers can be expected to be useful in any given pairwise combination. Moreover, as distribution of crossover can vary

substantially between different pairs of parents the actual genetic map position of any given marker may also differ (Beavis 1991). To facilitate utilization of the maps generated using the 90K SNP chip, Wang et al. 2014 created a consensus SNP map of wheat, based on the tested eight populations. In essence, this map provides average marker positions for all polymorphic markers of their study and may be used to coordinate maps generated for different populations.

As it was explained in an earlier chapter, total lengths of maps for each of the three populations here varied but more importantly, at times very few common markers were present in specific chromosome regions. For verified QTLs, that is for consistent associations between specific DNA markers and genome regions consistently showing up in replications, the consensus map was used to allocate those to specific regions and used DNA sequence data of the closest associated marker to blast against the wheat sequence survey on the URGI database (https://urgi.versailles.inra.fr/) and determine its actual location. In this fashion, relative locations of QTLs identified in this study can be compared to all previous results and can be verified in the future. This approach makes it possible to use even those DNA markers that were not polymorphic between two parents of a given population (hence they could not be placed on the population-specific genetic map) increasing the resolution of a mapping exercise.

This study identified 31 genomic regions associated with seminal root angle and seminal root number in three populations. Most of these regions were unique to specific populations and varied from year to year. This implies that these traits are far from simple, as proposed by Oyanagi (1991) and do not appear to be controlled by single loci.

It must be pointed out that compared to other studies on seminal root traits, the results presented here appear to be better supported by experimental data.

Using a single population of 103 doubled haploids Hamada et al. (2012) were unable to identify a QTL for seminal root angle; two QTLs for deep root ratio appeared on chromosomes 1B and 5D. Another QTL, for seminal root, was found on chromosome 5A. None of the regions consistently identified in this study appear to be located on chromosomes of Hamada et al (2012). In another study, Christopher et al. (2013) identified 12 QTLs for seminal root angle and number in a single mapping population of bread wheat consisting of 184 individuals. The QTLs for seminal root angle were located on chromosomes 2A, 3D, 5D, 6A, and 6B; those for seminal root number on chromosomes 1B, 3A, 3B, 4A, and 6A. While some chromosomes are the same as those identified in this study, none are on chromosomes verified as valid QTL in this study: 2DS, 6AL and 7BS for seminal root angle and 4BL for seminal root number. In another study Liu et al. (2013) again identified a total of 12 QTLs for seminal root angle and number. Seven of those, for seminal root angle were on chromosomes 1A, 2B, 3A, 3B, and 7D and five for seminal root number on 2B, 3B, 3D, 5A, and 7A. Again, there are some genome regions in common with this study but none appear to be are similar to our verified QTL.

Most studies employ a single mapping population. Beavis (1998) demonstrated that in populations numbering 100 progeny, the QTL effects were greatly overestimated, in populations with 500 progeny the QTL effects were slightly overestimated while populations with 1000 individuals produced estimates close to the actual magnitude of

QTL effects. That study highlighted the necessity for larger populations and the need for verification of QTL across populations. Beavis (1998) did not address the issue of mapping in parallel populations sharing common parents. To the best of our knowledge only a couple of studies made use of two or more populations in studying root system traits: Zhang et al. (2014) used three related recombinant inbred line populations with a single common parent and Kabir et al. (2015) used two unrelated populations. Zhang et al. (2014) identified QTLs for seminal root number on chromosomes 1D, 2A, 2B, 2D, 3A, 3B, 4A, 4D, 5A, 5D, 6A, 6B, and 7B. Several of these chromosomes also showed significant associations with this study, but the verified region on 4B was not among them. Similar to our results, individual QTL were almost always exclusive to each population. Kabir et al. (2015) identified QTLs for root number on 1B, 2A, 3A, 4A, 4D, and 7A and no QTL was consistent across the two populations. These observations suggest two explanations: either seminal root number is sensitive to environmental effects (that is, it's highly plastic) and many statistically significant associations detected in all studies are spurious, or this trait is controlled by a large number of genes, in different combinations in each parental line. No single locus appears to have a large dominant effect, perhaps with the exceptions of the loci on chromosomes 2DS and 4BL in our study.

Unfortunately the two studies of Zhang et al (2014) and Kabir et al (2015) did not investigate seminal root angle so we have no insight into how that trait behaved in both cases. Within our populations, the expression of seminal root angle QTLs were also highly dependent upon year and population, however, five QTLs were consistent across

both years (Table 2.4). One of those QTL, *QRA.ucr-2D,* was also verified within two of the three populations (Figure 2.10). This QTL accounted for the largest proportion of the phenotypic variation of all verified QTLs. This could potentially be due to the greater phenotypic difference between the two parents in the SC and SF populations which allow for greater detection of QTL (Tanksley 1993). Using relative genetic map distances this QTL appears to be the same QTL as identified by Bektas (2015) with a large effect upon other root traits such as deep root weight. Bai et al. (2013) also reported a QTL on chromosome 2D for seminal root biomass.

In other cases such as *QRA.ucr-6A* and *QRA.ucr-7B* the QTLs consistently appeared in the SC population in both years; however, they do not appear in other populations. Given the crossing pattern used in the development of the populations, and even with an assumption that the QTL donor (Sonora for the wide root angle in the SC population) carries the same allele as Foisy, segregation should have been observed in the CF population, but it was not. Perhaps this is because this QTL explains a small percentage of the total phenotypic variation and its effect is overshadowed, hence undetectable, by segregation of different allelic combinations within the SF population.

Interesting are some minor shifts in the suspected QTL positions between the years. In the CF population *qRA.ucr-5B* and *qRA.ucr-7A* varied more from one year to the next than any other QTL, and no association with common markers were detected, even though on the consensus map of Wang et al. (2014) all these associated markers fall within 10-20cM of one another. Because the effect of this specific genome region was reproducible it is deserving of further study. These shifts of QTL positions are often

associated with changes in the total amount of variation explained by the QTL between years. For example, *qRA.ucr-5B* in the CF population explained 9.40 % of the phenotypic variation in 2014 but 19.50% in 2015. These QTL appear verified as they produce significant effects in both years, however, their effects were not detected in the other two populations. This may be an effect of considerable plasticity of the characters measured, illustrating technical difficulties in precise phenotyping. On the other hand, this may hint at the existence of closely linked loci within the same family, each with a minor effect on the total expression of the character, and minor variation within the environment from one year to the next may cause shifts in the locus/loci responsible, thus changing marker associations in the region. These examples could potentially be shedding light on the plasticity of QTL for seminal root angle in light of environmental cues. New techniques such as the clear pot method proposed by Richard et al. (2015) may provide less variability by reducing the experimental error.

Unraveling the genetics of seminal root angle in wheat may prove to be a longer road than in other crops like rice. Uga et al. (2013) identified *DEEPER ROOTING 1* (*DRO1*) as a gene controlling the gravitropic response of roots and thus the angle of root growth. Higher expression of *DRO1* caused roots to grow more downward and when introduced into a shallow rooted cultivar it improved grain yield under drought by enabling access to water deeper in the soil profile. It is likely simpler to study quantitative traits like seminal root angle within the smaller diploid genome of rice. Although there is synteny between rice and wheat within the region where *DRO1* was identified, QTL in that region were not identified. Until recently rice was the closest relative of wheat that

had information about seminal root angle genetics. However, researchers interested in barley have now begun to study seminal root traits as well (Robinson et al. 2016). Using the clear pot method demonstrated by Richard et al. (2015) they were able to identify seven QTLs for seminal root angle and number (root angle, two QTLs; root number, three QTLs). Using cross species analysis they were able to identify 10 common genes underlying root trait QTLs in barley, wheat, and sorghum. Perhaps as seminal root angle is unraveled in barley, a closer relative to wheat than rice, it will provide insights which may aid in our understanding of wheat seminal root trait genetics.

Correlation of seminal root angle and number

Seminal root angle and number appear to be interrelated and both appear to be related to seed weight (Table 2.2). In the SC population root number and angle are negatively correlated so that seeds with more seminal roots have narrower angles and vice versa. This correlation explained 22% and 30% of the variation seen in 2014 and 2015 respectively. In the SF population seminal root number and seed weight were positively correlated so that heavier seeds tended towards a higher number of seminal roots. That correlation explained 36% and 45% of the variation in 2014 and 2015 respectively. In the CF population all these characters are correlated where seminal root number is positively correlated with seed weight and seminal root angle is negatively correlated with number and weight. The correlation between root number and seed weight explained 46% of the variation in 2014 and 2015, seminal root number and angle explained 24% and 33% in 2014 and 2015 respectively, and the correlation of seminal

root angle and seed weight explained 23% and 35% of the variation. These results show that in the CF population a significant amount of the variation can be explained by these interactions. This is interesting in that those two parents have more similar seminal root angles (Figure 2.2). Since seed weight explained a significant amount of the variation for root number and angle it could mean loci for seminal root angle or number in CF are actually seed weight QTLs.

The only way to ascertain which character is actually monitored is to map QTLs for seed weight and test their associations with those found for seminal root angle and number. In the SC and SF population no QTL for seed weight was similar to that mapped for root angle and number. However, in the CF population two QTLs for seed weight were in similar positions to QTLs for root angle and number (Figure 2.11a, b). The QTL for seed weight on chromosome 1B clearly overlaps with the QTL for root number on 1B, each sharing common markers in both years. Of the QTLs mapped for root number in CF this QTL on 1B was the only one observed in both years. For the QTL on 5B there are not any overlapping markers for the root angle QTL and seed weight QTL, however, the QTLs for root angle on 5B shift from one year to the next making this region suspect and deserving of further inquiry. Of the QTLs for root angle in the CF population the QTL on 5B explained the greater portion of variation seen in the population over two years. Since so much variation is explained by the interaction of seed weight with angle and number it is not a major leap to assume this region could be associated with seed weight and inadvertently associated with root angle. Given those results, coupled with the correlation

analysis, it seems that seed weight is a major factor, if not the major factor, in the CF population giving rise to most differences in seminal root traits.

These interactions between seed weight, seminal root angle and seminal root number indicate the high complexity of root traits. The nature of these interactions has not been tested but it appears plausible that when five seminal roots are initiated they occupy greater space at the developing point of the embryo than when only three roots are initiated. This may force the inner pair of roots more downward, thus reducing the angle between them and explain why more seminal roots is correlated with narrower angles of growth and why those with less seminal roots have a tendency toward wider angles. Additionally, heavier seeds are correlated with higher seminal root numbers which then may influence the association of seed weight with seminal root angle. Perhaps this argument is overly simplistic, and it does not begin to explain why these three characters are correlated only in some populations, and why the levels of interaction change from one population to the next. Another explanation could be linkage of loci for individual traits which could make them difficult to tease apart. In any case, these correlations underscore the complexity of these traits and call for further dissection of each trait and their interactions, so that actual genetic effects are studied. Those interactions could lend new dimensions of complexity when considering the inheritance of seminal root angle and number. These findings also provide new information for considerations when designing future projects centered on these traits. Another point to be made is that QTLs in other studies should be further verified and looked at again

through this perspective. As far as we know, other studies did not map QTLs for seed weight when an interaction was observed with seminal root traits.

Conclusions

Previous studies on root characteristics in wheat have identified numerous genomic regions associated with seminal root angle and number. Few of those presumed QTLs have been verified in other populations and/or even in multiple years. No one so far has looked at seminal root angle and seminal root number QTLs in the same experiment, and specifically in a set-up with built-in verification system, such as the one employed here.

Overall, seminal root angle is proving to be much more complicated than originally proposed and will likely be more difficult to unwind than in other crops such as rice and maize. The interactions between seminal root angle, seed weight and seminal root number should be further explored; each adding a new dimension to the complexity of the trait. It would be prudent for future studies to incorporate related and unrelated populations, or association mapping, to verify consistent biologically significant QTLs across genetic backgrounds. Such material should then be studied under field conditions to understand how laboratory-measured QTLs and phenotypes are important in a given environment. This would help to better understand the relevance of seminal root angle in improving and maintaining grain yields for wheat in a shifting climate.

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Table 2.1: Mean, maximum and minimum values, ANOVA results, and broad sense heritability for (a) seminal root angle and (b) number measured at 7 days after germination in the SC, SF, and CF populations

(a)	Population					
	SC		SF		CF	
Year	2014	2015	2014	2015	2014	2015
Mean	69.66	83.05	76.40	83.62	63.14	71.26
Max	105.05	118.43	107.65	114.25	80.77	92.83
Min	27.63	28.74	45.36	55.14	35.35	43.27
P genotypes[a]	0.00	0.00	0.00	0.00	0.00	0.00
CV (%)	14.66	15.77	13.87	11.55	16.13	16.46
H^2 (%)	70.18	68.41	64.88	60.53	52.30	56.55
LSD (P<0.05)	14.19	21.05	14.73	13.42	14.16	16.31
(b)						
Mean	4.62	4.05	4.38	4.53	4.74	4.39
Max	5.75	5.44	5.66	5.75	5.50	5.38
Min	3.00	3.00	3.00	2.63	3.00	2.62
P genotypes[a]	0.00	0.00	0.00	0.00	0.00	0.00
CV (%)	9.69	13.62	11.89	10.96	8.39	11.96
H^2 (%)	63.10	63.92	46.96	49.96	57.05	53.53
LSD (P<0.05)	0.62	0.89	0.72	0.69	0.55	0.73

[a] Significance of the difference between genotypes in the given population

Table 2.2: Pearson's correlation coefficients for root number (RN), seed weight (SW), and seminal root angle (RA) in all populations for 2014 and 2015

Population	Year	Trait	RN	SW	RA
SC	2014	RN	1.000		
		SW	0.0996	1.000	
		RA	-0.2217*	-0.0473	1.000
	2015	RN	1.000		
		SW	0.2878	1.000	
		RA	-0.3049*	-0.0668	1.000
SF	2014	RN	1.000		
		SW	0.3641*	1.000	
		RA	-0.0959	-0.0996	1.000
	2015	RN	1.000		
		SW	0.4465*	1.000	
		RA	-0.1514	-0.1451	1.000
CF	2014	RN	1.000		
		SW	0.4621*	1.000	
		RA	-0.2422*	-0.2343*	1.000
	2015	RN	1.000		
		SW	0.4643*	1.000	
		RA	-0.3258*	-0.3457*	1.000

*p-value significant (α=0.01)

Table 2.3: Summary of genomic regions associated with for seminal root angle (RA) and number (RN) in 2014 and 2015

Pop.	Trait	Year	Chrom.	Pos. (cM)[a]	Left Marker	Right Marker	LOD	PVE (%)[b]	ADD[c]
SC	RA	2014	2D	113	2Dx_79444	2Dx_77420	21.31	25.99	6.95
		2014	3B	84	3B_6987	3B_36611	8.50	8.29	3.93
		2014	3B	172	3B_49558	3B_47344	4.93	4.57	-2.91
		2014	6A	151	6A_72189	6A_55084	7.31	7.04	3.62
		2014	6B	201	6B_4107	6B_5378	7.14	6.78	3.56
		2014	6D	17	6D_35645	6D_44501	5.59	5.36	3.16
		2014	7B	84	7B_3402	7B_61463	7.54	7.32	3.69
		2015	2D	112	2Dx_32130	2Dx_79444	12.75	21.42	7.33
		2015	4A	92	4A_78420	4A_56921	6.03	9.53	4.89
		2015	5A	29	5A_65358	5A_12124	5.56	10.18	5.05
		2015	6A	155	6A_55084	6A_21174	4.85	7.21	4.26
		2015	7B	84	7B_3402	7B_61463	5.19	7.70	4.39
	RN	2014	5B	142	5B_2723	5B_66420	3.37	9.32	0.16
		2014	5B	208	5B_630	5B_70323	5.70	16.73	-0.22
		2015	4A	161	4A_61756	4A_34374	7.61	17.32	-0.25
		2015	7A	78	7A_11533	7A_42098	4.97	10.91	-0.20
SF	RA	2014	2D	52	2Dx_10084	2Dx_77420	8.35	22.96	6.06
		2015	2D	36	2Dx_78609	2Dx_10084	5.81	13.24	4.43
		2015	5B	154	5B_40362	5B_8581	3.30	7.11	3.28
		2015	6B	80	6B_606	6B_84	3.29	7.06	3.24
		2015	7B	63	7B_67435	7B_12657	3.30	7.17	3.31
	RN	2014	4B	91	4B_12434	4B_13349	5.76	15.81	-0.19
		2015	4B	91	4B_12434	4B_13349	4.61	12.77	-0.18

Table 2.3 Continued.

CF	RA	2014	5B	58	5B_7678	5B_9324	3.79	9.40	2.90
		2014	6A	92	6A_71395	6A_35951	3.92	9.63	2.90
		2014	7A	84	7A_9696	7A_38343	4.01	9.98	-2.95
		2015	5B	50	5B_7411	5B_3193	8.28	19.50	5.22
		2015	7A	99	7A_6878	7A_29223	3.89	3.37	-3.39
	RN	2014	1B*	56	1B_5588	1B_3191	2.25	7.81	0.12
		2015	1B	54	1B_63003	1B_53084	3.74	8.68	0.15
		2015	6B	6	6B_66298	6B_49223	3.60	8.41	0.15
		2015	7D	137	7D_76924	7D_15372	3.63	8.43	0.15

[a]Genetic position rounded to the nearest centiMorgan (cM)

[b]Phenotypic variation explained by the region

[c]Estimated additive effect of the region

[*]Not significant based upon the LOD threshold determined by permutation when $\alpha = 0.05$, but highly suggestive of a QTL

Table 2.4: Summary of QTL that were consistent over multiple years and verified within and/or across populations. In the QTL ID the abbreviation RA denotes seminal root angle and RN seminal root number. Uppercase "Q" in the name signifies strong verification of the QTL and lowercase "q" was used to name QTL that warrant further investigation. The parent contributing the allele for wider seminal root angle is listed.

Pop.	**QTL ID**	**Chrom.**	**Pos. (cM)[a]**	**Marker[b]**	**LOD[c]**	**PVE (%)[d]**	**ADD[e]**	**Parent**
SC, SF	*QRA.ucr-2D*	2DS	43.69	2Dx_10084	11.82	20.73	5.81	Sonora
SC	*QRA.ucr-6A*	6AL	131.70	6A_55084	6.08	7.13	3.94	Sonora
SC	*QRA.ucr-7B*	7BS	58.17	7B_3402	7.17	9.01	4.42	Sonora
CF	*qRA.ucr-5B*	5BL	n/a	n/a	6.04	14.45	8.12	CBdeM
CF	*qRA.ucr-7A*	7A	n/a	n/a	3.95	6.68	-3.17	Foisy
SF	*QRN.ucr-4B*	4BL	74.62	4B_12434	5.19	14.29	-0.19	Foisy

[a]Genetic position of associated marker based upon Wang et al. (2014) consensus map
[b]Closest associated marker to the QTL
[c]Average LOD score over the two years
[d]Average phenotypic variation explained by the QTL over two years
[e]Average estimated additive effect of the QTL over two years

Figure 2.1: Measuring seminal root angles. For each plant the angle between the first pair of seminal roots was measured at approximately 3 cm from the seed using image analysis software (ImageJ). Yellow arrows show the first pair of seminal roots and arcs represent the angle measured between those roots.

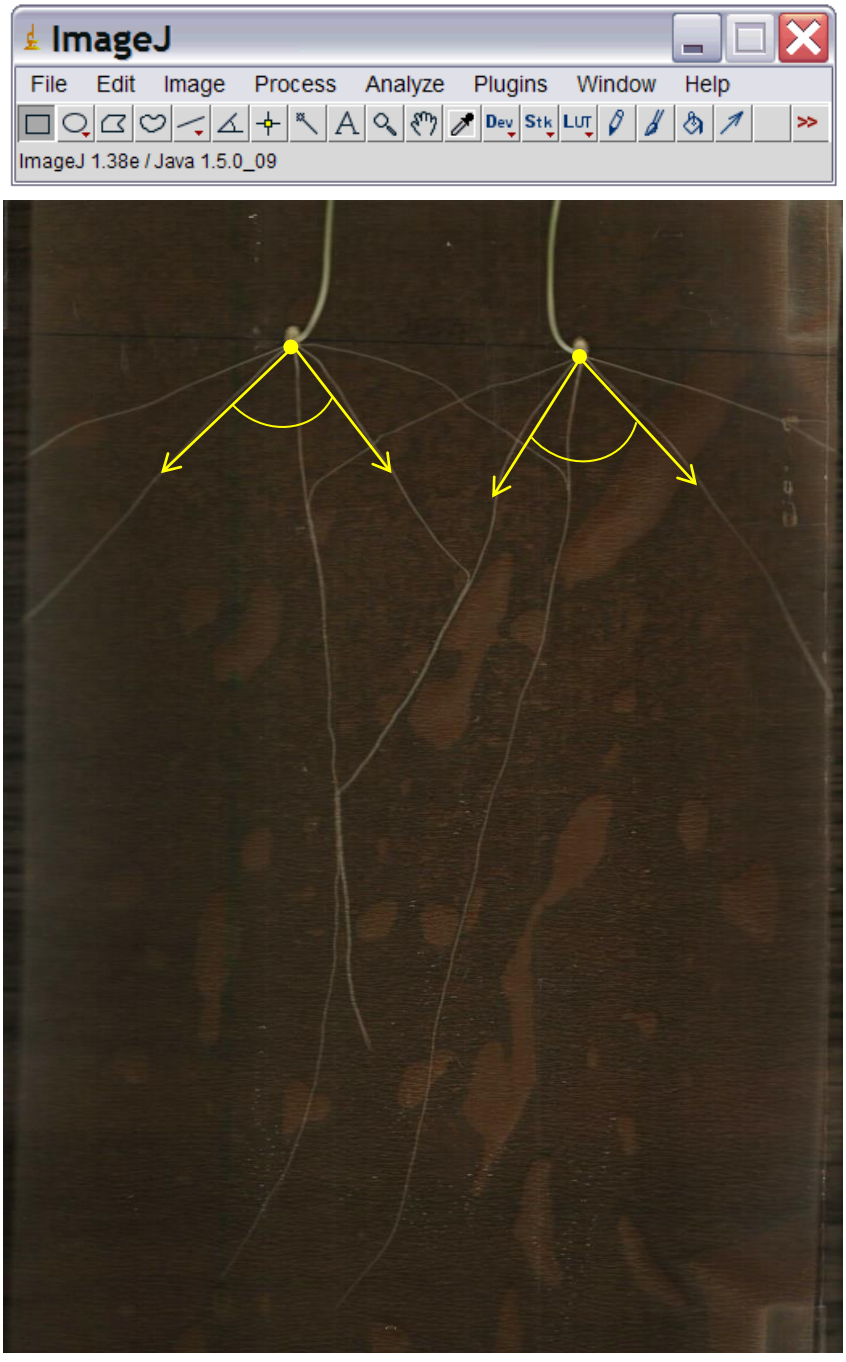


Figure 2.2: Frequency distribution of seminal root angle (°) in the SC, SF, and CF populations. Red, green and black arrows highlight the group that parents Sonora, Foisy, and CBdeM fall into respectively.

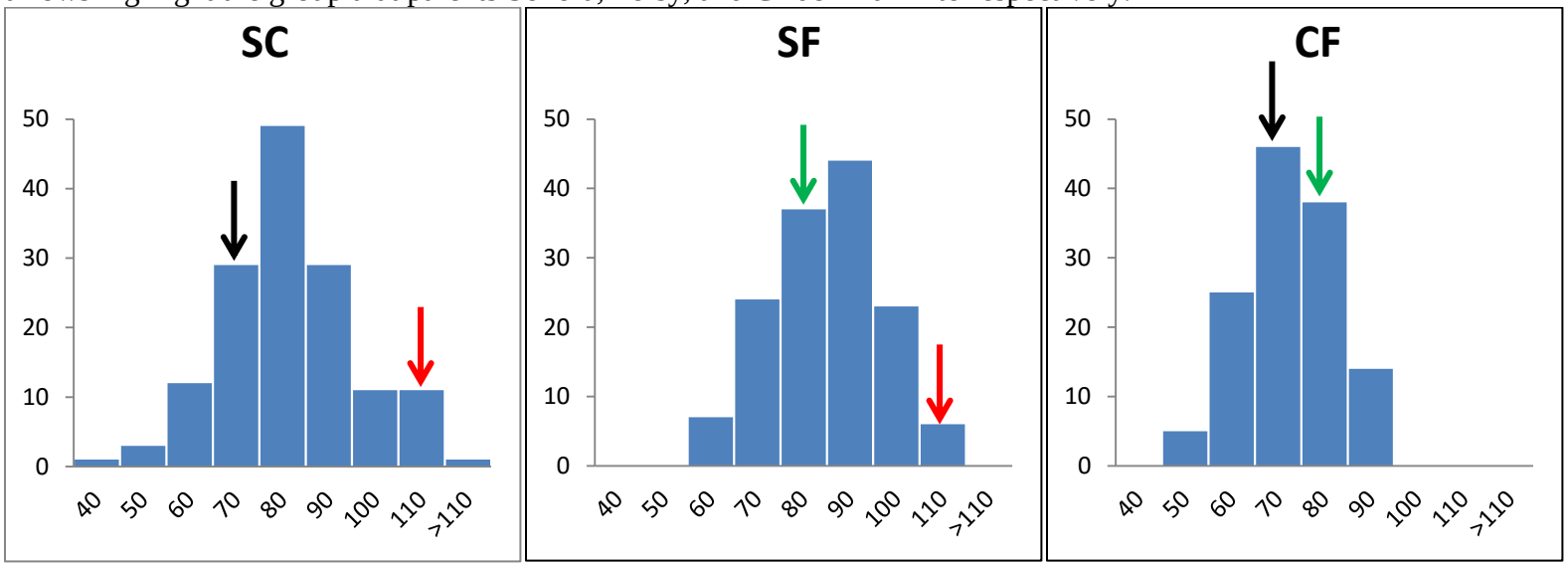


Figure 2.3: QTL for seminal root angle on chromosome arm 2DS in the SC population detected with IciMapping using the composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where $\alpha = 0.05$. The green line is the QTL in 2014 and red is 2015.

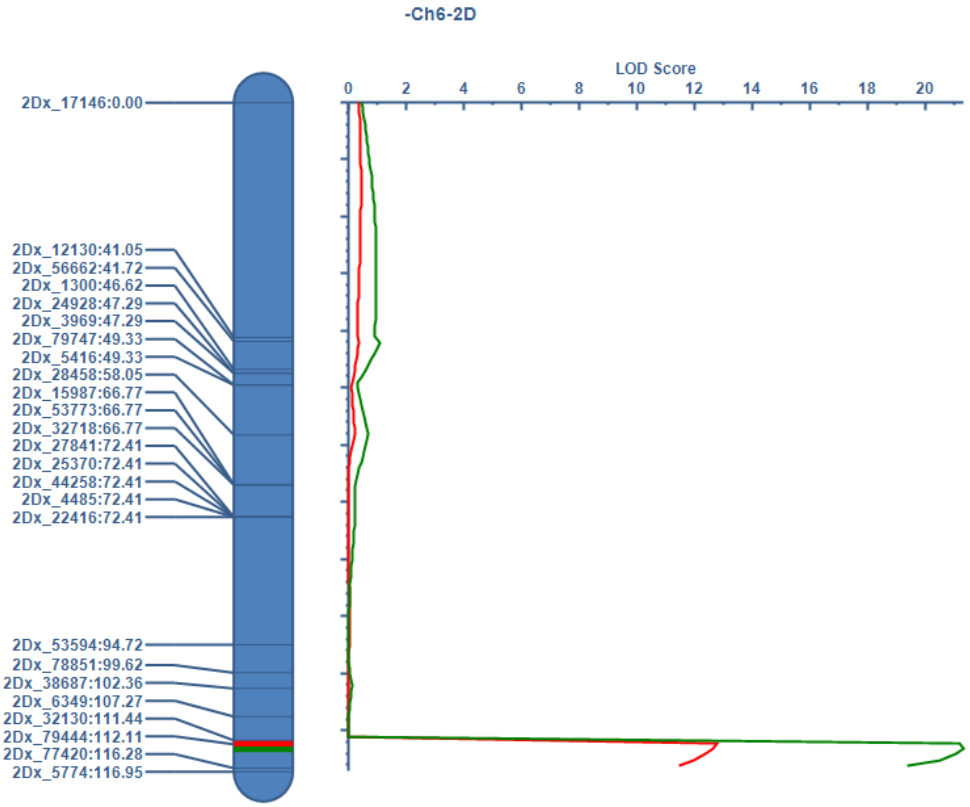


Figure 2.4: QTL for seminal root angle on chromosome arm 6AL in the SC population detected with IciMapping using the composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where $\alpha = 0.05$. The green line is the QTL in 2014 and red is 2015.

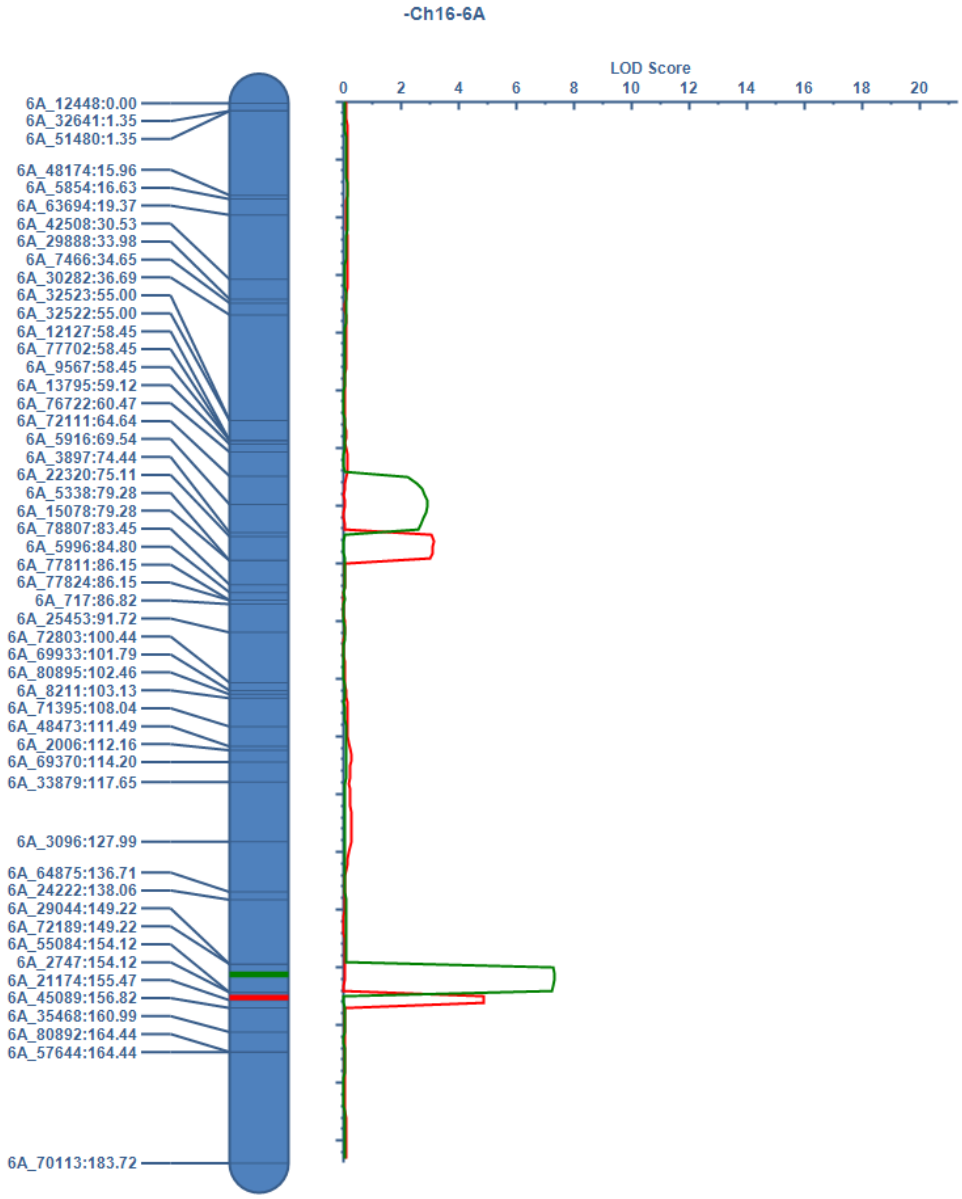


Figure 2.5: QTL for seminal root angle on chromosome arm 7BS in the SC population detected with IciMapping using the composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where $\alpha = 0.05$. The green line is the QTL in 2014 and red is 2015.

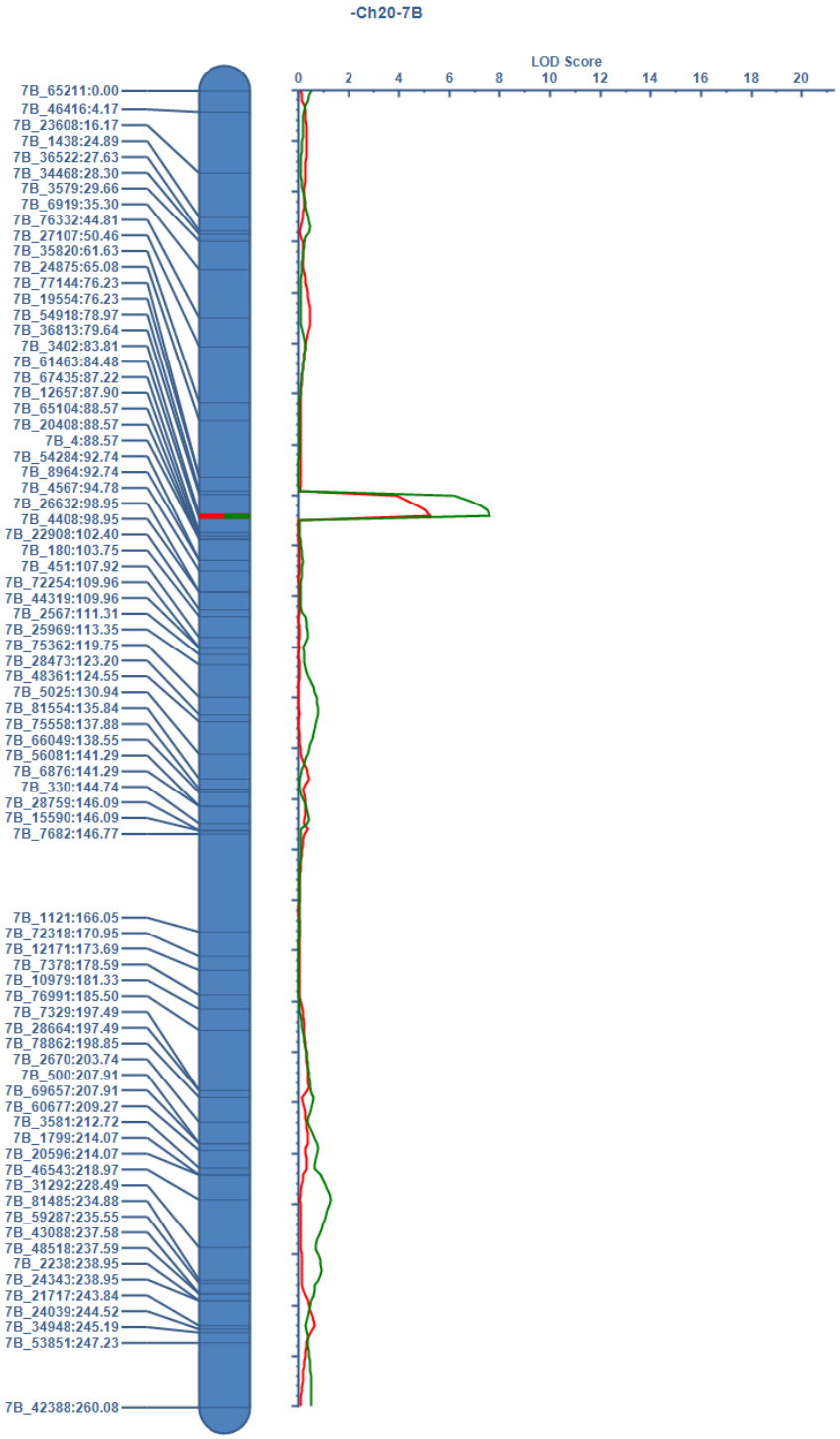


Figure 2.6: QTL for seminal root angle on chromosome arm 2DS in the SF population detected with IciMapping using the composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where $\alpha = 0.05$. The red line is the QTL in 2014 and green is 2015.

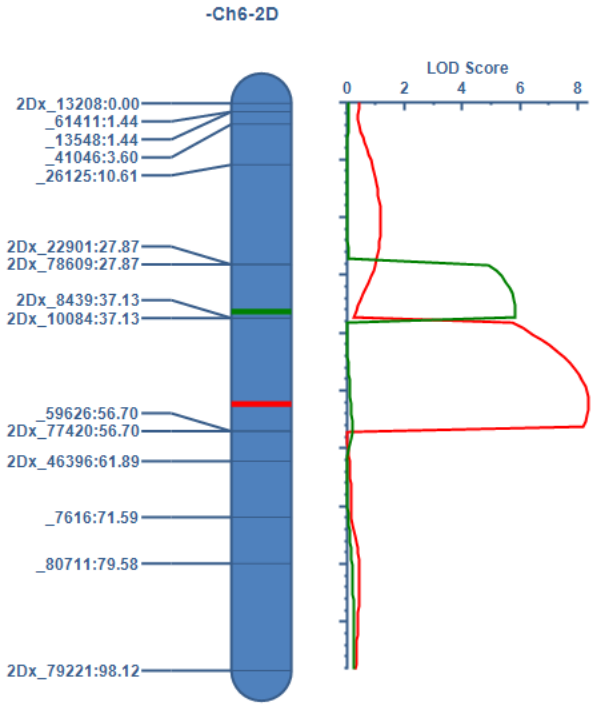


Figure 2.7: QTL for seminal root angle on chromosome arm 5BL in the CF population detected with IciMapping using the composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where α = 0.05. The red line is the QTL in 2014 and green is 2015.

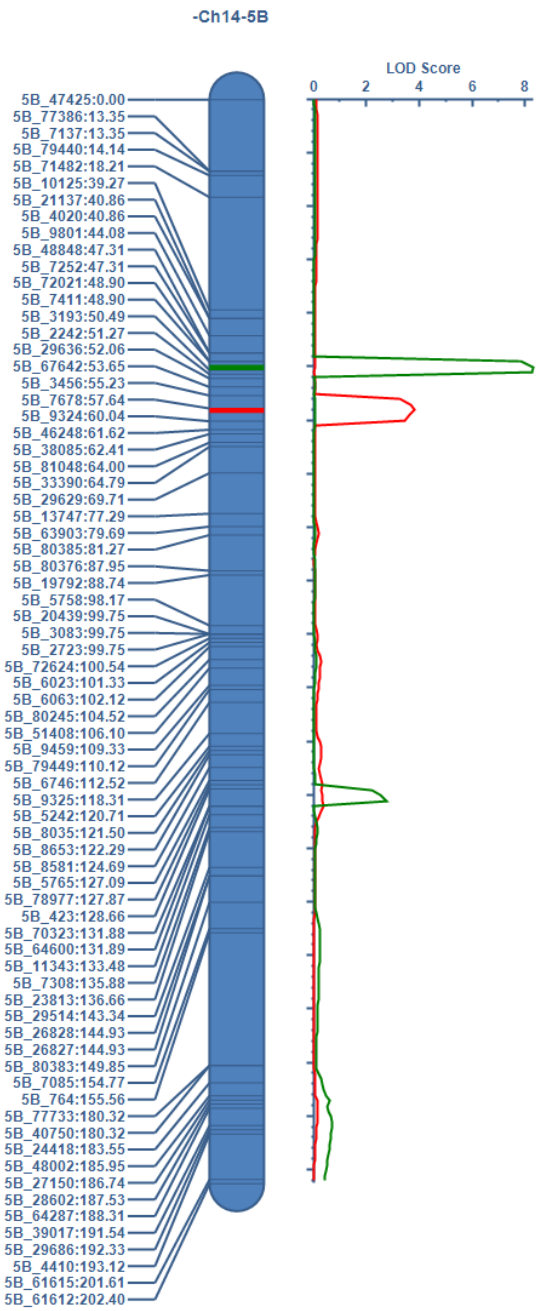


Figure 2.8: QTL for seminal root angle on chromosome 7A in the CF population detected with IciMapping using the composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where $\alpha = 0.05$. The red line is the QTL in 2014 and green is 2015.

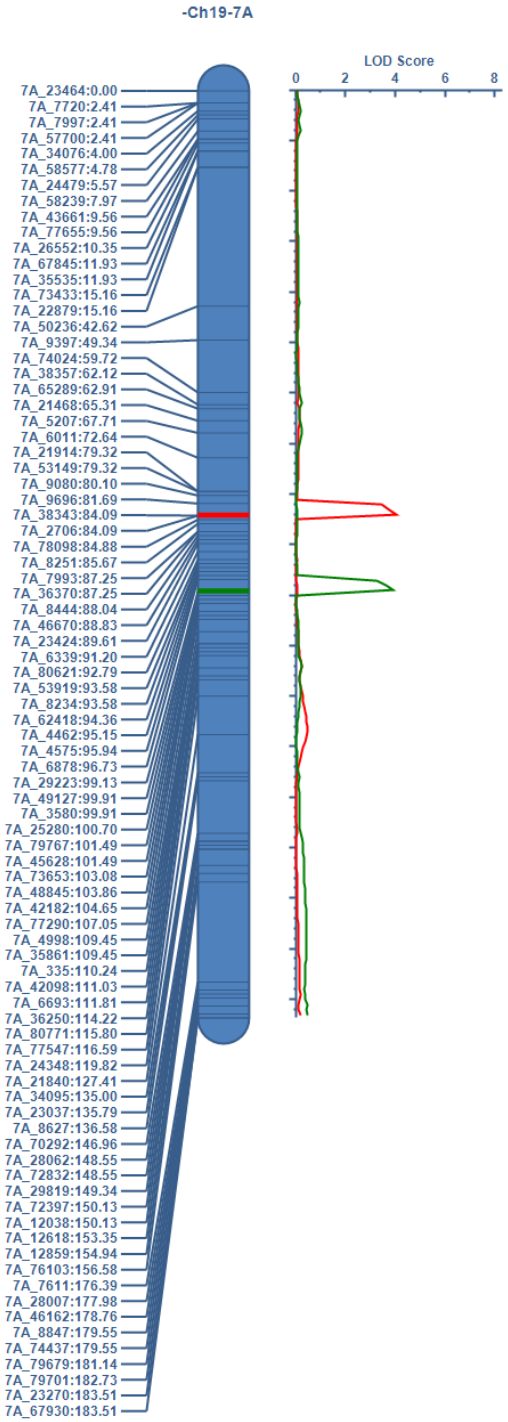


Figure 2.9: QTL for seminal root number on chromosome arm 4BL in the SF population detected with IciMapping using the composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where $\alpha = 0.05$. The red line is the QTL in 2014 and green is 2015.

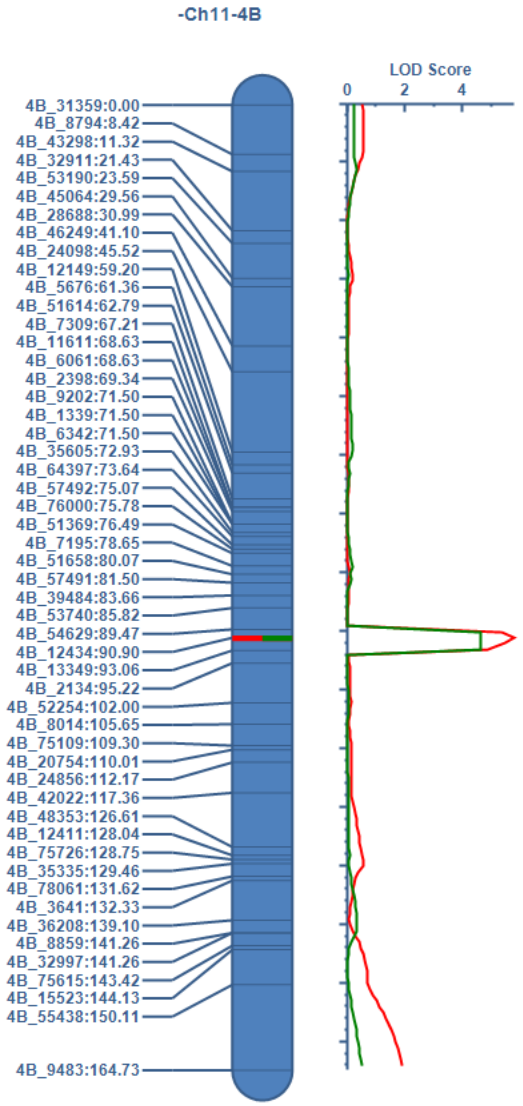


Figure 2.10: Comparison of QTLs for seminal root angle on chromosome arm 2DS in the (a) SF and (b) SC population detected with IciMapping using the composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where $\alpha = 0.05$. The arrow designates the marker shared in common between the two populations providing a good source of verification of the QTLs as being the same.

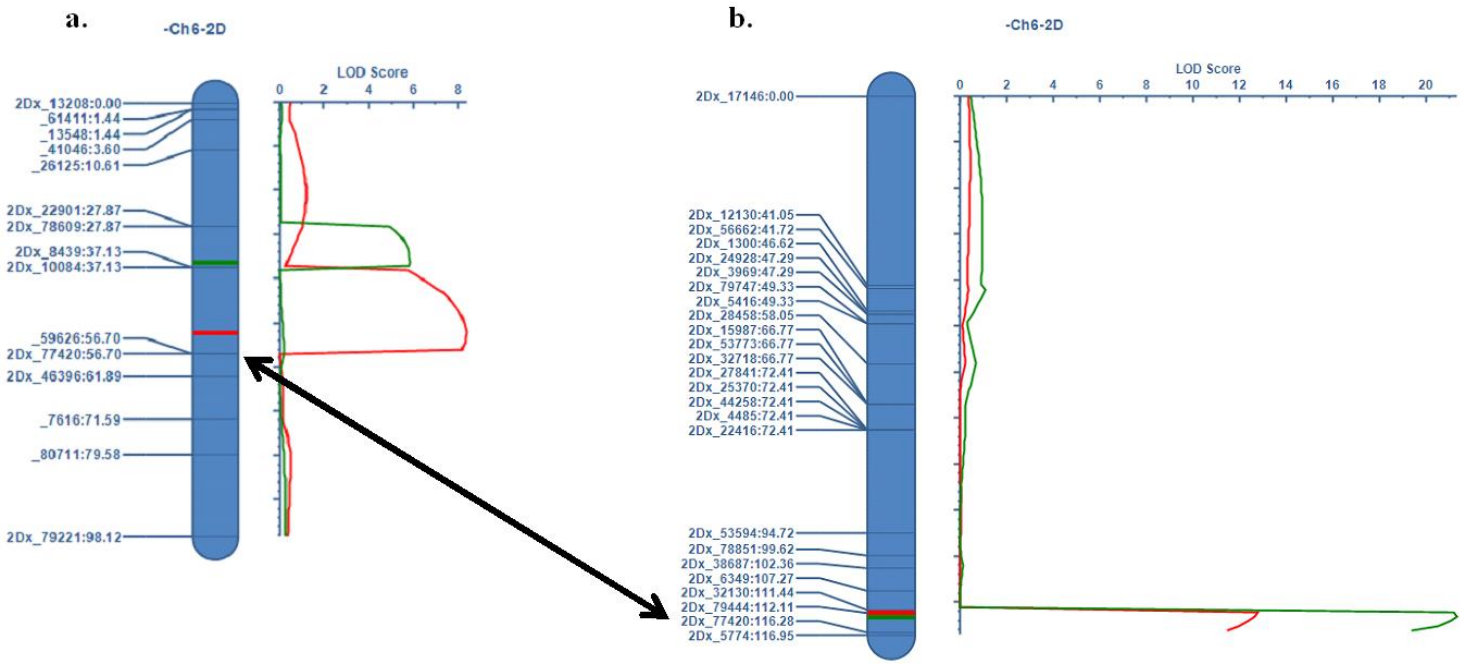


Figure 2.11: (a) QTLs for seed weight (SW) and their overlap with QTLs for root number (RN) in the CF population. Green and red are QTLs for SW in 2014 and 2015, respectively. Blue and purple are QTLs for RN in 2014 and 2015, respectively. (b) QTLs for seed weight (SW) and their similarity to QTLs for root angle (RA) in the CF population. Green and red are QTLs for SW in 2014 and 2015, respectively. Purple and yellow are QTLs for RA in 2014 and 2015, respectively.

(a)

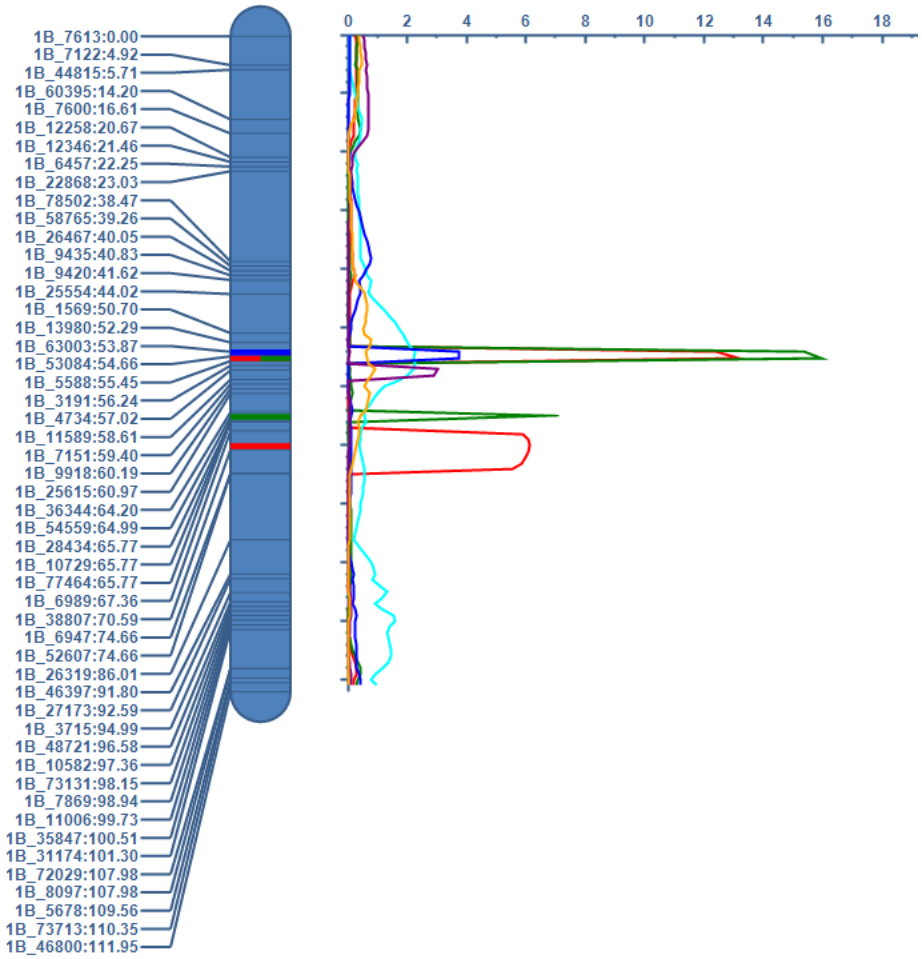
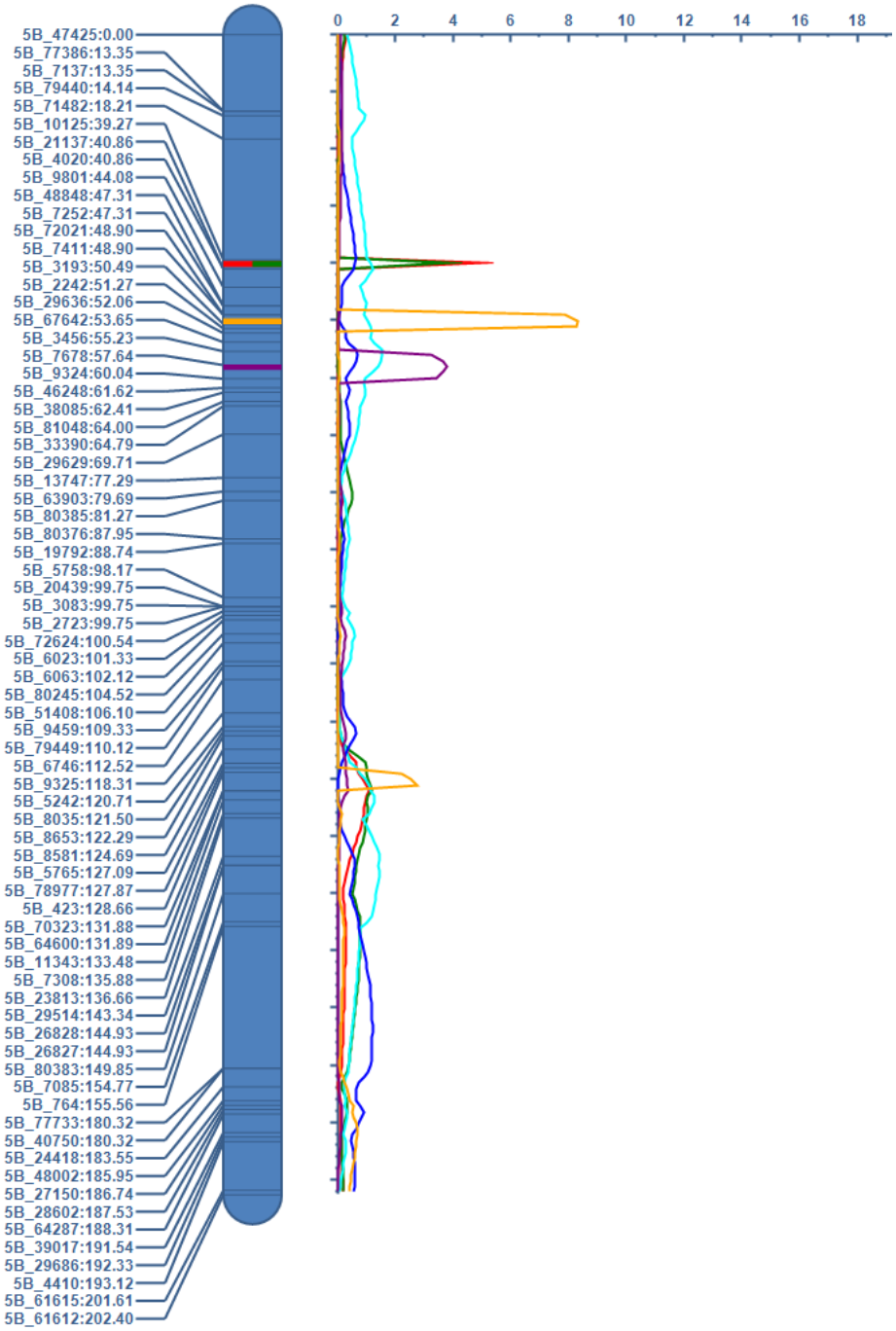


Figure 2.11 Continued.

(b)



Chapter 3

Insights into the Genetics of Root Biomass and its Relationship to Shoot Biomass

Abstract

Previous research has shown that increased root biomass increases grain yield under limited water environments. It has also been demonstrated that root biomass is genetically controlled while also being influenced by the environment. These facts make studying root biomass in agricultural crops, such as wheat, a valued venture. However, before efforts are made towards altering the root systems of crops it would be prudent to understand the relationship between shoots and roots. Root system traits are notoriously challenging to study and pose many obstacles to researchers interested in unraveling the genetics behind those traits. Three related populations of doubled haploids were used to map quantitative trait loci (QTLs) controlling root biomass. Root and shoot biomasses were directly related to one another and heading date. Tests have also shown the correlation values of shoot and root biomass increased in proportion to the length of any given test. In 2016 an average of 84.1% of the variation seen in all three populations was explained by the positive correlation between shoot and root biomass. Additionally, a large set of data from multiple experiments was analyzed to gain some insights into the general relationship between shoot and root biomass in wheat. In total 6,353 data points were included to create scatter plots of root and shoot biomass and grain yield. For a

higher resolution, two cultivars were tested under various regiments designed to generate variation in root and shoot biomass. The analysis demonstrates that while a general correlation exists between root biomass, shoot biomass, and grain yield, individual cultivars may substantially deviate from it.

Introduction

Since the green revolution, semi-dwarf high yielding wheat cultivars have become a standard in commercial production. The semi-dwarf character of wheat lead to a three-fold increase in grain yield and provided food security for developing countries (Borlaug 2007). These green revolution wheats were selected for under high-input farming practices which led to a decrease in root biomass (Waines and Ehdaie 2007). A greater understanding of root traits and how those traits relate to whole plant strategies may enable breeders to increase yields under drought conditions (Comas et al. 2013). This understanding can only come by actively studying the root system in a controlled environment and until the relationship of root and shoot traits is better understood we cannot determine how to improve a plants ability to be productive in a fluctuating environment.

Roots absorb water and nutrients while also anchoring the plant to the soil. The shoots utilize those resources for photosynthesis and are the site of sexual reproduction. All these functions must work together in coordination for the plant to thrive within its environment. In general plants maintain a fairly strict harmony between shoot and root biomass partitioning (Davidson 1969; Makela and Sievanen 1987). However, during different growth and developmental stages the partitioning of biomass does fluctuate. In the early stages of growth resource allocation and biomass accumulation is focused towards the roots but that shifts considerably as the plant reaches flowering with the major part of photosynthates directed to the shoots (Evans and Wardlaw 1976; Gregory

1994). These general principles were supported by Frageria (1992) who demonstrated that the root-to-shoot ratio in wheat, as well as other crops, decreased as plants advanced in age. For these reasons it is essential to understand what effect any changes to these general principals may have upon yield within wheat and other crops as well.

Increased root biomass increases grain yield under limited or rain-fed environments (Palta et al. 2011). This is likely due to the ability of a larger root system to absorb water and nitrogen from the soil; an added benefit is reduced leaching and agricultural run-off (Waines and Ehdaie 2005). What remains unclear is if increasing root biomass will continue to increase grain yields. This issue has been touched upon in wheat by Maheepala et al. (2015) leaving plenty of room for further inquiry and testing. In their findings, as root biomass increased beyond a certain threshold it negatively impacted grain yields, perhaps due to increased costs of maintaining a large root system. This point appears worthy of detailed study as it may point to which root system traits will be beneficial in a given environment and how they might impact yields if conditions become favorable. Perhaps a large root system may be beneficial when water is limited; however, will a large root system remain an advantage if water becomes sufficient? These types of questions need to be answered before efforts are made toward modifying crop root system traits.

Understanding the genetics of root system traits does not have to wait until we have all the answers. Since root traits are highly plastic and regulated by a number of small-effect loci it will likely take some time to unravel these complex traits. Yaseen and Malhi (2011) reported that wheat genotypes varied significantly in their allocation of dry

matter in roots and shoots. Frageria (1992) also demonstrated that root dry weight was genetically controlled as well as being influenced by the environment. Currently, the neglect of selecting for root system traits is mainly due to the difficulty of measuring root system traits of field-grown plants (Richards 2008). As our understanding of root system genetics is improved new tools such as genetic markers associated with desired traits can be utilized by breeders for marker-assisted breeding efforts.

Materials and methods

Growing systems

Two systems were used in the experiments described below. One consisted of tubes fitted with a plastic sleeve filled with sand. A filter paper covering two holes punctured at the bottom of the sleeve allowed for drainage. In the standard system water is applied to the top of the tube (Ehdaie and Waines 2006). In a modified version an additional plastic sleeve was fitted to allow for water to be delivered from the bottom up. Tubes were brought to water holding capacity before planting. The second system consisted of pots lined with a plastic sleeve, filled with sand and four holes punctured in the plastic for drainage. Pots were brought above water holding capacity and allowed to drain for 24 hours before being planted. Peters Excel fertilizer (21-5-20 N-P-K, www.scottspro.com) was injected to the irrigation water at a 1:100 ratio. Every irrigation event in the experiments included this ratio of fertilizer.

In both systems, after the experiments were terminated plants were processed in the same manner. Shoots were separated from the roots by harvesting them at the sand level. Roots were then washed clean of sand. Shoots and roots were dried for 72 hours in a forced air drier at 80°c at which point they were weighed for total biomass.

Mapping populations

Three doubled haploid bread wheat populations were used to measure total root biomass at various stages of growth. Parents were selected for their root biomass among 16 spring wheat landraces and modern cultivars tested by Waines et al. (2012). Cv. Sonora ranked among the highest, Foisy was intermediate, and CBdeM had a low total root biomass. These parents have other contrasting phenotypes for traits such as drought tolerance, plant height, days to heading, awn type, and seminal root angle. Detailed information about genotyping, linkage mapping and general descriptions of each population can be found in the first chapter of this dissertation. Populations Sonora x CBdeM (abbreviated as SC), Sonora x Foisy (SF), and CBdeM x Foisy (CF) have 146, 141, and 128 lines respectively.

In 2013 the three parental lines were evaluated for root biomass in 80cm tubes using the standard method. These were grown for 30 days and 60 days as preliminary evaluations at two growth stages. Plants were grown in a factorial design with four replications. Doubled haploid progeny were grown in one gallon pots with four replications in a randomized complete block design for 21 and 28 days in 2014 and 2015 respectively. The 2016 experiments included 100 randomly selected progeny from each

population with three replications due to constraints on greenhouse space. Plants were grown until heading (40+ days). Plants were watered as needed to keep the sand at water holding capacity for the duration of the experiments.

Combined data analysis

To gain a broad perspective about the relationship between shoot and root biomass, data from multiple experiments were combined. Data sets from experiments running for a similar duration were combined to fit into a similar scale. Following these criteria three sets of data were created. The first set was created from the data of the tradeoff experiments described below and data kindly provided by Dr. Harun Bektas (Bektas 2015). The second set is from the data collected during the allelic variation experiments (Chapter 4) and the mapping population data collected in 2016 mentioned bellow. The third set is from the 2014 and 2015 mapping population experiments described below. Raw values for individual were used to create scatter plots fitted with a Loess smoothing curve with an alpha of 0.75 with a quadratic degree using statistical analysis software, Statistix 10 (http://www.statistix.com/).

Tradeoff experiments

Cvs. Pavon 76 and Yecora Rojo are semi-dwarf wheats developed by the International Center for Maize and Wheat Improvement (CIMMYT) that have been used extensively as standards in root studies conducted at the University of California, Riverside by Dr. J. Giles Waines and his co-workers. With a substantial amount of

foundational data for these cultivars they were chosen to run a set of experiments aimed at observing the trade-offs and relationships between shoot and root growth.

Plants were grown to maturity in the modified tube system and the pot system. Seeds of Pavon 76 and Yecora Rojo were imbibed for 24 hours before planting. For each cultivar two treatments plus a control were run in a factorial design with two replications in fall 2015 and winter 2016. The control was given 500mL of water daily, from the top down. The fist treatment received water from the bottom only starting at two weeks of growth. Water from the bottom was kept at the furthest point at which the roots reached within the sand as visible through the clear plastic sleeve of two check tubes. As the roots grew into the water profile the water level was continuously dropped until the roots reached the bottom of the tube. The second treatment received water from both the top and the bottom daily. Water from the bottom was maintained at 50cm to prevent roots from growing deeper and 500mL of water was added each day from the top.

The pot experiments had three replications in fall 2015 and winter 2016 setup in a factorial design having three treatments and a control. Each pot, for both cultivars, was kept at water holding capacity until three different phenological stages: booting, heading and anthesis at which point water was cut entirely. Treatments are termed as drought-at-booting, drought-at-heading, and drought-at-anthesis. After that point, any plant showing severe water stress was given water to prevent death. This point was determined when leaves began to wilt and curl beyond mild symptoms. The control was given ample water to maintain the sand at water holding capacity throughout the experiment.

During the experiments days to booting, days to heading, days to anthesis, and days to maturity were recorded. Days to booting was recorded at the point when the flag leaf emerged. Days to heading was recorded at the point when the head split the boot. Days to anthesis was recorded at the point when the first anthers became dehiscent. Days to maturity was recorded at the point when the grain was ready to harvest. At maturity the total number of tillers and total number of fertile tillers were counted. Heads were harvested and the shoots were cut at the soil surface to separate them from the roots. Shoot biomass is reported without grain yield included in the total biomass. Heads were threshed to record grain yield for each plant. For the tube experiments, root length was measured and root biomass above 30 cm was separated from the root biomass bellow 30 cm and weighed separately.

Statistics and quantitative trail locus mapping (QTL)

The analyses of variance (ANOVAs) for traits in each experiment were based on mean values of the experimental units and considered significant where $p \leq 0.05$. Genotype means were used for LSD all-pairwise comparisons where $\alpha = 0.05$. In experiments involving the mapping populations, broad sense heritability (H^2) and Pearson's correlation coefficients for shoot and root biomass were calculated on a mean basis across four replications.

Genomic regions associated with root and shoot biomass were detected by the software package IciMapping (http://www.isbreeding.net) (Li et al. 2007) using linkage maps for the mapping populations as described previously (Chapter 1) and the mean

value of four seedlings of each genotype from four replicates in 2014 and 2015; in 2016 the mean value of three seedlings from three replications was used. The composite interval mapping method with a step of 1cM was used and the threshold for QTL detection was determined using 1000 permutations where $\alpha = 0.05$. Markers in the linkage maps were renamed using the index number provided by Wang et al. (2014) preceded by the chromosome designation.

Results

Mapping populations

At 30 days of growth Foisy had the largest root biomass with a mean of 0.48 grams, Sonora ranked second with 0.42 grams and CBdeM had the lowest with 0.25 grams (Figure 3.1). These means were not significantly different from one another ($p < 0.05$). At 60 days, parental lines had mean root biomasses of 4.08, 3.83, and 2.11 for Foisy, Sonora, and CBdeM respectively, with Foisy and Sonora being significantly different from CBdeM ($p < 0.05$). All populations of DH progeny showed significant variation for shoot and root biomass in all three years demonstrating transgressive segregation in all cases (Figures 3.2 to 3.7).

The SC population had shoot biomasses ranging from 0.39 to 1.12, 0.62 to 1.42, and 4.94 to 19.06 grams in 2014, 2015, and 2016 respectively. Sonora had an average shoot biomass of 0.72, 1.03, and 11.13 grams and average root biomass of 0.31, 0.30, and 2.23 grams in 2014, 2015, and 2016 respectively. CBdeM had an average shoot biomass

of 0.71, 0.94, and 14.57 grams and average root biomass of 0.27, 0.33, and 3.38 grams in 2014, 2015, and 2016 respectively. Coefficient of variation (CV) for shoot biomass was 26.84, 22.60, and 35.21 in 2014, 2015, and 2016 respectively. Critical values for comparison were 0.27, 0.31 and 6.42 to 7.89 grams in 2014, 2015, and 2016 respectively. The broad sense heritability for shoot biomass was 0.30, 0.16, and 0.47 in 2014, 2015, and 2016 respectively. Root biomasses ranged from 0.15 to 0.60, 0.16 to 0.61, and 0.86 to 5.21 grams in 2014, 2015, and 2016 respectively. CV for root biomass was 33.77, 40.25, and 42.95 in 2014, 2015, and 2016 respectively. Critical values for comparison were 0.14, 0.18, and 1.87 to 2.99 grams in 2014, 2015, and 2016 respectively. Broad sense heritability was 0.36, 0.28, and 0.55 in 2014, 2015, and 2016 respectively. In the 2016 ANOVA analysis the homogeneous group format for all pairwise comparison couldn't be used because of the pattern of significant differences. Genotypes could only be compared in single pairs at once giving a range in critical values for comparison.

The SF population had shoot biomasses ranging from 0.25 to 0.63, 0.34 to 0.94, and 3.96 to 12.93 grams in 2014, 2015, and 2016 respectively. Sonora had an average shoot biomass of 0.43, 0.59, and 8.50 grams and average root biomass of 0.17, 0.24, and 2.58 grams in 2014, 2015, and 2016 respectively. Foisy had an average shoot biomass of 0.45, 0.49, and 7.38 grams and average root biomass of 0.15, 0.28, and 1.67 grams in 2014, 2015, and 2016 respectively.CV for shoot biomass was 25.14, 27.32, and 40.55 in 2014, 2015, and 2016 respectively. Critical values for comparison were 0.15, 0.23 and 4.78 to 7.18 grams in 2014, 2015, and 2016 respectively. The broad sense heritability for shoot biomass was 0.30, 0.07, and 0.44 in 2014, 2015, and 2016 respectively. Root

biomasses ranged from 0.09 to 0.34, 0.12 to 0.47, and 0.87 to 6.93 grams in 2014, 2015, and 2016 respectively. CV for root biomass was 37.07, 50.65, and 51.34 in 2014, 2015, and 2016 respectively. Critical values for comparison were 0.08, 0.18, and 1.68 to 2.52 grams in 2014, 2015, and 2016 respectively. Broad sense heritability was 0.24, 0.08, and 0.49 in 2014, 2015, and 2016 respectively. In the 2016 ANOVA analysis the homogeneous group format for all pairwise comparison couldn't be used because of the pattern of significant differences. Genotypes could only be compared in single pairs at once giving a range in critical values for comparison.

The CF population had shoot biomasses ranging from 0.27 to 0.75, 0.84 to 2.27, and 4.20 to 19.58 grams in 2014, 2015, and 2016 respectively. CBdeM had an average shoot biomass of 0.52, 1.60, and 11.10 grams and average root biomass of 0.20, 0.56, and 2.12 grams in 2014, 2015, and 2016 respectively. Foisy had an average shoot biomass of 0.51, 1.53, and 11.37 grams and average root biomass of 0.23, 0.54, and 2.20 grams in 2014, 2015, and 2016 respectively.CV for shoot biomass was 23.51, 22.85, and 27.15 in 2014, 2015, and 2016 respectively. Critical values for comparison were 0.17, 0.51 and 4.88 to 6.00 grams in 2014, 2015, and 2016 respectively. The broad sense heritability for shoot biomass was 0.26, 0.34, and 0.69 in 2014, 2015, and 2016 respectively. Root biomasses ranged from 0.09 to 0.38, 0.24 to 1.05, and 0.87 to 6.25 grams in 2014, 2015, and 2016 respectively. CV for root biomass was 30.42, 40.82, and 34.88 in 2014, 2015, and 2016 respectively. Critical values for comparison were 0.10, 0.33, and 1.35 to 1.65 grams in 2014, 2015, and 2016 respectively. Broad sense heritability was 0.30, 0.22, and 0.70 in 2014, 2015, and 2016 respectively. In the 2016 ANOVA analysis the

homogeneous group format for all pairwise comparison couldn't be used because of the pattern of significant differences. Genotypes could only be compared in single pairs at once giving a range in critical values for comparison.

A total of 12 QTLs were detected in the three populations (Table 3.1). The nine QTLs for shoot biomass were on chromosomes 1B, 5B, 5D, 6A, 6B, and 7B. The three QTLs for root biomass were on chromosomes 2A and 5D. No QTLs were consistent over multiple years in any of the three populations.

In the SC populations correlation analysis showed that shoot and root biomass were positively correlated with 77.5, 45.7, and 86.3 % of the variation being explained by this relationship in 2014, 2015, and 2016 respectively. Correlation analysis for heading dates in 2016 against shoot and root biomass explained 89.2 and 83.2 % of the variation for those traits in the SC population respectively. Shoot and root biomass were also positively correlated in the SF population with 58.5, 36.8, and 78.2 % of the variation being explained by this relationship in 2014, 2015, and 2016 respectively. Correlation analysis for heading dates in 2016 against shoot and root biomass explained 87.2 and 72.7 % of the variation for those traits in the SF population respectively. The same correlation was seen in the CF population with 58.8, 61.0, and 87.5 % of the variation being explained by that relationship in 2014, 2015, and 2016 respectively. Correlation analysis for heading dates in 2016 against shoot and root biomass explained 90.0 and 78.5 % of the variation for those traits in the CF population respectively.

Combined data analysis and tradeoff experiments

In total 6,353 data points were included to create the scatter plots for the combined data analysis of the relationship between root and shoot biomass (Figure 3.8). The first set included 1,243 data points (Figure 3.8a). The second set had 1,342 points (Figure 3.8b). The third set had a total of 3,768 data points (Figure 3.8c). In the first set of data, root mass versus shoot mass and grain yield were plotted as those experiments went until maturity. All other experiments were concluded earlier having only shoot and root biomass collected.

Data for the fall and winter 2016 tradeoff experiments were not significantly different so the data were combined. In the pot experiments Pavon 76 did not show significant differences between drought treatments and the control for days to booting, days to heading, or days to anthesis with means of 49.1, 54.3, and 59.1 days respectively. However, days to maturity showed significant differences between the treatments and the control, with means of 92.8 and 137.5 days respectively. The total number of tillers was also significantly different between the treatments and the control with means of 9.3 and 34.3, respectively. The total number of fertile tillers showed significant differences within treatments as well as between treatments and the control. Plants experiencing drought-at-booting had means of 5.5 fertile tillers, those at-heading and at-anthesis 8.3 fertile tillers, and the control had means of 32.2 fertile tillers per plant. For shoot biomass, the treatments showed significantly less biomass than the control with a mean of 11.9 grams versus 36.0 grams for the control. Root biomass was significantly different between plants receiving drought-at-anthesis versus those at-booting and plants receiving drought-

at-heading had a similar mean when compared to either of the other treatments. Mean root biomass for plants receiving drought at booting, heading, and anthesis were 3.5, 4.9, and 5.1 grams respectively. All drought treatments were significantly less than the control which had a mean total root biomass of 7.1 grams. Grain yield for the drought treatments were significantly less than the control which had a mean yield of 51.3 grams. Plants receiving drought-at-heading yielded the second highest with a mean of 12.0 grams and both the drought-at-booting and drought-at-anthesis treatments yielded the lowest with a mean of 6.3 grams.

Similar to Pavon 76 in the pot experiments, Yecora Rojo did not show significant differences between drought treatments and the control for days to booting, days to heading, or days to anthesis with means of 29.6, 36.7, and 41.6 days, respectively. Days to maturity showed a significant difference between the treatments and the control with means of 77.9 and 106.5 days, respectively. The total number of tillers for the drought treatments was 6.6 which was significantly lower than the control with 9.5 tillers per plant. Treatments showed significant differences with the drought-at-booting and drought-at-anthesis treatments having means of 5.0 and 7.0 fertile tillers respectively. Plants receiving drought-at-heading were intermediate between the other two treatments with a mean of 6.0 tillers per plant which was not significantly different the other treatments. All treatments had lower means than the control which had 8.8 fertile tillers per plant. For shoot biomass all treatments were similar with a mean of 3.1 grams which was significantly different from the control of 4.7 grams. The control had the highest root biomass with 2.6 grams per plant and the drought-at-anthesis treatment had the next

largest root biomass with 1.9 grams per plant. Both the booting and heading treatments had a mean of 1.3 grams per plant. Grain yield also showed significant difference within the treatments and between the treatments and the control. The control yielded 11.3 grams per plant, the drought-at-anthesis yielded 5.7 grams per plant, and the drought-at-heading treatment yielded 4.7 grams per plant which was not significantly different from the drought-at-anthesis treatment or the drought-at-booting treatment, and the drought-at-booting treatment yielded the lowest with 3.6 grams per plant.

In the tube experiments Pavon 76 did not show significant differences between the treatments and control for days to booting, days to heading, days to anthesis, or days to maturity with means of 55.13, 60.6, 64.8, and 125.2 respectively. The number of tillers was significantly different within the treatments and between the treatments and control. The shallow treatment had the largest number of tillers with 28.5 tillers per plant, the control ranked second with 18.3 tillers, and the deep treatment had a mean of 5.0 tillers per plant. Of the total number of tillers 27.3, 16.3, and 4.0 were fertile tillers for the shallow, control, and deep treatments, respectively with all being significantly different. Shoot biomass varied significantly dependent upon treatment with means of 38.2, 20.1, and 5.7 grams for the shallow, control, and deep treatment respectively. Root biomass above 30 cm followed the same trend with means of 6.3, 2.7, and 0.94 grams for the shallow, control, and deep treatment respectively. Root biomass bellow 30 cm showed no significant difference between treatments and the control with a mean of 2.1 grams. Total root biomass showed significant differences within the treatments and between treatments and the control. The shallow treatment had the largest total root biomass with 8.3 grams

per plant, next was the control with 5.3 grams which did not vary significantly from the shallow treatment or the deep treatment which had a mean of 2.8 grams per plant. In the control and deep treatment roots reached to the bottom of the 1 meter tube and as planned the shallow treatment did not grow much into the anaerobic volume of sand saturated with water bellow 50cm. The mean root length in the shallow treatment was 57.3 cm. Both treatments and the control varied significantly for yield with means of 54.1, 29.5, and 7.5 grams per plant for the shallow, control, and deep treatment respectively.

Yecora Rojo did not show significant differences in the tube experiments for days to booting, days to heading, or days to anthesis with means of 36.5, 42.4, and 46.58 days respectively. For days to maturity, there was a significant difference within treatments and between the deep treatment and the control. The deep treatment had a mean of 79.0 days to maturity while the control and shallow treatment had a mean of 116.1 days. The total number of tillers was significantly different for the control and deep treatment which had 10.8 and 2.0 tillers per plant respectively. The shallow treatment was intermediate and not significantly different from either with a mean of 6.5 tillers. The number of fertile tillers was not significantly different between the control and shallow treatment having 9.3 and 6.0 fertile tillers per plant respectively. The deep treatment did vary significantly from those with a mean of 2.0 fertile tillers per plant. Shoot masses of the two treatments were not significantly different due to large variances of the groups with means of 3.2 and 0.76 grams for the shallow and deep treatment, respectively. Both treatments were significantly different from the control which had a mean shoot biomass of 7.3 grams per plant. Root biomass above 30 cm was significantly different within treatments and

between treatments and the control with means of 1.8, 1.1, and 0.2 grams per plant. Root biomass between the treatments and the control were significantly different but treatment has a similar mean biomass of 0.3 grams per plant. The control had a mean of 4.5 grams per plant. Total root biomass showed the same results with the shallow and deep treatment having a non-significant difference of 1.2 and 0.7 grams per plant, while the control was significantly different having a mean of 6.3 grams of total root biomass per plant. In the control and deep treatment, roots reached the bottoms of the 1 meter tubes and the shallow treatment had a mean root length of 45 cm. Grain yield was significantly different within treatment and between treatments and the control. The control yielded a mean of 11 grams per plant, the shallow treatment yielded 6.1 grams, and the deep treatment yielded 2.1 grams per plant.

Cvs. Pavon 76 and Yecora Rojo were significantly different for all traits except root length and root mass bellow 30 cm. The contrasts between cultivars created such large difference in biomass that results have to be displayed on graphs with different scales for the pot and tube systems as shown in Figure 3.9 and 3.10 respectively. Scatter plots for root biomass in grams plotted against shoot biomass and yield in grams for cultivars Pavon 76 and Yecora Rojo are shown in Figure 3.11. Data shown are combined from both systems used in the tradeoff experiments. The scatter plots are also in different scales due to large differences between cultivars.

Discussion

Genetic variation for root biomass

Despite the fact that all three mapping populations demonstrated significant differences for root and shoot biomass no QTLs were verified in multiple years and populations. The obvious difficulty is the large CV values recorded for all experiments. The variation observed within genotypes was so large that the critical values for comparison were also very high. This level of variation makes association between genetic markers and phenotypes unreliable. In general, as the duration of experiments increased the broad sense heritability increased. This likely explains why most QTLs that were reported are from experiments being run for a longer duration, with the majority coming from 2015 and 2016. Of those QTL reported many are within the same region as loci reported for heading time in wheat, some of which were reported in chapter 1 of this dissertation. The only QTLs for root biomass came from the SC population in 2016 and those QTLs are the same as those reported for shoot biomass the same year which are also heading time QTLs. It is clear that root and shoot biomass are directly related to heading dates for that population. This is supported by the results from the correlation analysis which showed that in general as the experiments were run for longer durations the correlation value of shoot and root biomass also increased. In 2016 an average of 84.1% of the variation seen in all three populations was explained by the positive correlation between shoot and root biomass and heading date explained an average of 88.8 and 78.1 % of the variation seen for those traits respectively. It is obvious for these

populations that shoot and root biomass are largely determined by heading time loci. To better understand if heading time, shoot biomass, and root biomass can be untangled populations with similar phenology for traits like heading time should be used (Cane et al. 2014).

Root and shoot biomass relationships

The combined data analysis indicates that initially shoot and root biomass increase proportionately, however, at a certain point as root biomass continues to increase shoot biomass begins to decrease. The same is true when root biomass is plotted against yield (Figure 3.8a). The same trend was observed by Maheepala et al. (2015).

To analyze this relationship in more detail Pavon 76 and Yecora Rojo were grown in the tradeoff experiments in two systems under multiple treatments designed to generate variation in root and shoot biomass. Testing these cultivars in different systems and under different water regimes was intended to verify any consistent relationships that might be observed. These two cultivars have been extensively tested before and are known for drastic contrasts in terms of root and shoot biomass as well as phenological traits such as heading date and plant height. Any trends observed in such contrasting material may permit some generalization about root and shoot biomass relationships.

Pavon 76 and Yecora Rojo clearly have different relationships in root biomass and yield while having similar shoot and root biomass relationships (Figure 3.11). In both cultivars there is an almost linear correlation for root and shoot biomass. That relationship is still consistent with the general observation provided by the combined data

analysis since both are placed at the beginning of the trend line. However, in Pavon 76, as root biomass increases, grain yields continue to increase; in Yecora Rojo as root biomass continues to increase grain yields begin to drop. Pavon 76 demonstrates a trend directly opposite to that observed from all combined data; Yecora Rojo closely follows the general trend as the combined data. These two cases imply that while possibly some general trend exists between root biomass, shoot biomass, and yield, individual lines or cultivars may deviate from it in a substantial way.

A notable feature of Pavon 76 is its ability to maintain relatively higher root biomass even under stressed conditions; on the other hand Yecora Rojo shows a greater decrease in root biomass as stress increased (Figure 3.9 and 3.10). This might have been the main factor that created this contrast in root biomass and yield relationships. At this point it is not certain what caused the contrasts observed in the two cultivars but the issue seems worth further study. Additional tests could include cultivars having similar phenology. If these were to show similar reactions to drought, perhaps some generalizations could be made based upon phenology.

Conclusions

Previous work has shown that traits such as root biomass and root-to-shoot ratio are genetically and environmentally controlled. The results presented here make it obvious that these traits are highly complex and in many cases environmental effects are so high that drawing out differences between genotypes becomes impossible. However,

accumulated data permit some general observations about root and shoot relationships in wheat. Generally, as shoot biomass increases root biomass increases as well but only to a certain point; beyond it further increase in root biomass is associated with a negative impact on grain yields. This is possibly due to an imbalance of resource allocation and the high cost of maintaining the large root system. Those generalizations cannot be blindly applied to all genotypes which makes it important to verify if individual cultivars or lines follow those general observations.

Perhaps a new system for studying root biomass could overcome some of the challenges facing scientists interested in studying the root system. It is clear that a controlled environment is necessary in these preliminary studies to identify QTLs. Here greenhouses were used which provide a sort of controlled environment; maybe growth chambers would be better suited to the task. Overall, the suggestion of Dorlodot et al. (2007) to study "process based" traits such as tropism and growth rate rather than "static" traits such as length and biomass seems to be well warranted.

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Table 3.1: Summary of QTL detected for shoot and root biomass in the SC, SF, and CF populations in 2014, 2015, and 2016. For shoot and root biomass the parent contributing the allele for higher biomass is listed.

Population	Trait	Year	Chrom.	Position (cM)[a]	Left Marker	Right Marker	LOD	PVE (%)[b]	ADD[c]	Parent
SC	SM	2015	7B	131	7B_5025	7B_81554	3.98	10.92	-0.05	CBdeM
		2016	5D	72	5D_4695	5D_17310	17.73	67.97	-2.96	CBdeM
		2016	5D	156	5D_63558	5D_5776	5.04	14.51	1.29	Sonora
		2016	6B	26	6B_66298	6B_47396	4.50	12.30	1.19	Sonora
	RM	2015	2A	164	2A_38933	2A_8706	3.50	10.58	0.03	Sonora
		2016	5D	80	5D_502	5D_77786	11.00	44.68	-0.74	CBdeM
		2016	5D	159	5D_63558	5D_5776	4.36	15.94	0.43	Sonora
SF	SM	2016	5B	100	5B_5758	5B_41910	3.78	14.22	0.83	Sonora
CF	SM	2014	5B	143	5B_23813	5B_29514	3.94	13.26	-0.03	Foisy
		2015	1B	57	1B_3191	1B_4734	3.43	8.90	0.08	CBdeM
		2015	6B	58	6B_8557	6B_9354	3.29	8.60	0.08	CBdeM
		2016	6A	70	6A_9169	6A_3692	3.25	13.94	-1.40	Foisy

[a]Genetic position rounded to the nearest centiMorgan (cM)
[b]Phenotypic variation explained by the QTL
[c]Estimated additive effect of the QTL

Figure 3.1: Total root biomass for mapping population parents measured at 30 days and 60 days after germination in spring 2013. Groups are designated with different letters (LSD $p<0.05$).

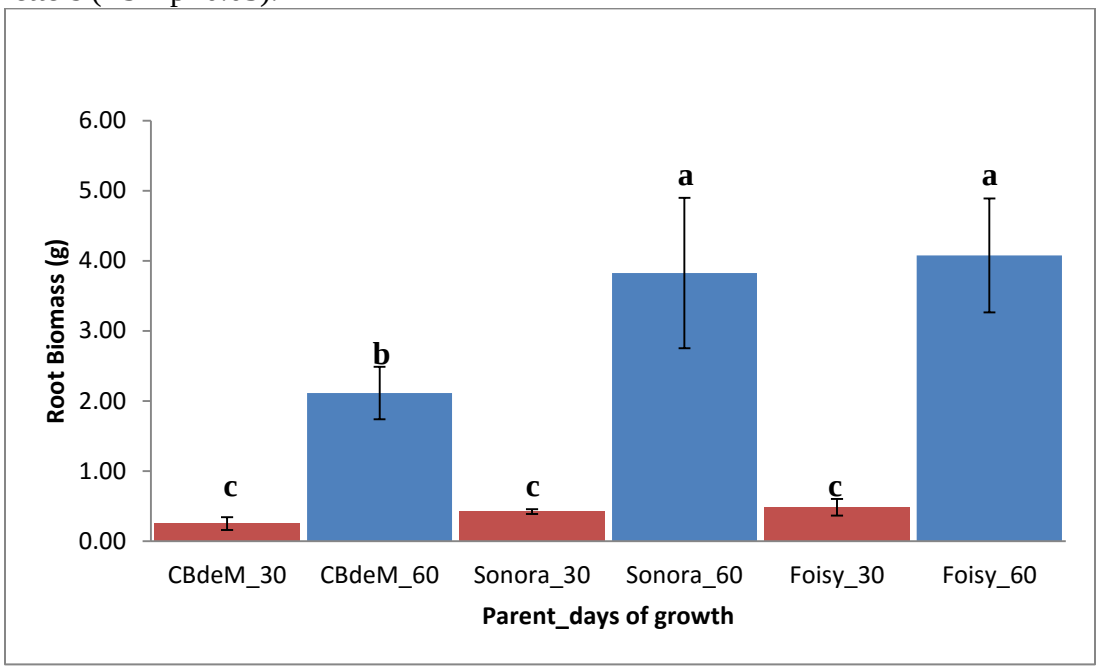
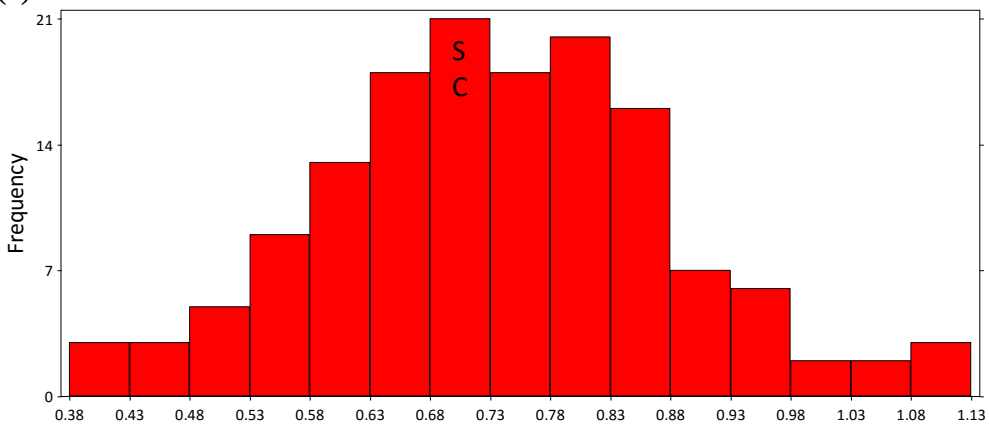
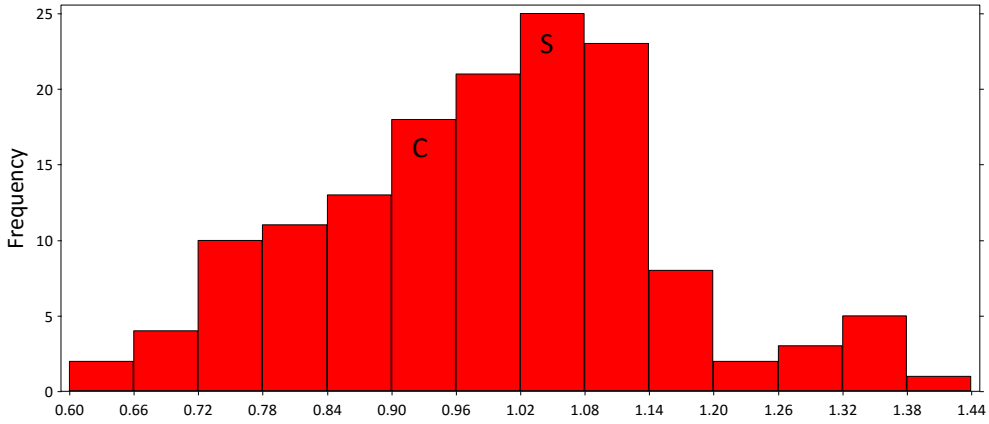


Figure 3.2: Histograms showing the frequency distribution of the SC populations for shoot biomass in grams for (a) 2014, (b) 2015, and (c) 2016 experiments. Note that all histograms are in different scales due to differences created from the various duration of each experiment. Groups in which the parents are found are marked by "S" for Sonora and "C" for CBdeM.

(a)



(b)



(c)

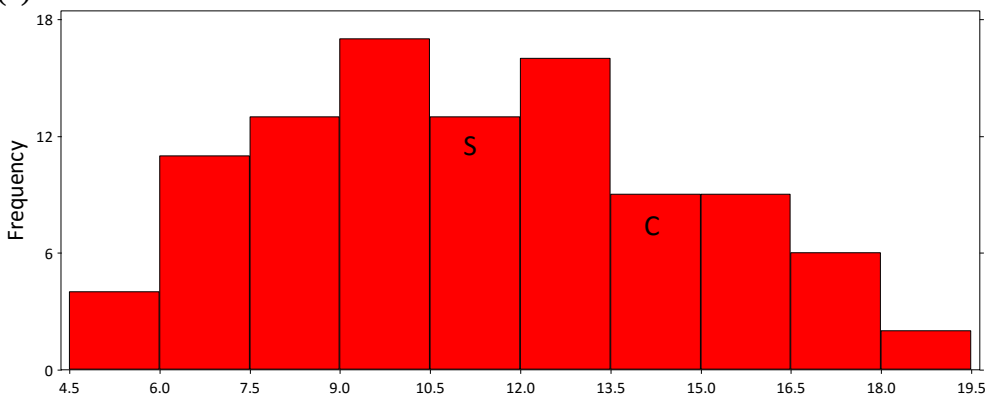


Figure 3.3: Histograms showing the frequency distribution of the SC populations for root biomass in grams for (a) 2014, (b) 2015, and (c) 2016 experiments. Note that all histograms are in different scales due to differences created from the various duration of each experiment. Groups in which the parents are found are marked by "S" for Sonora and "C" for CBdeM.

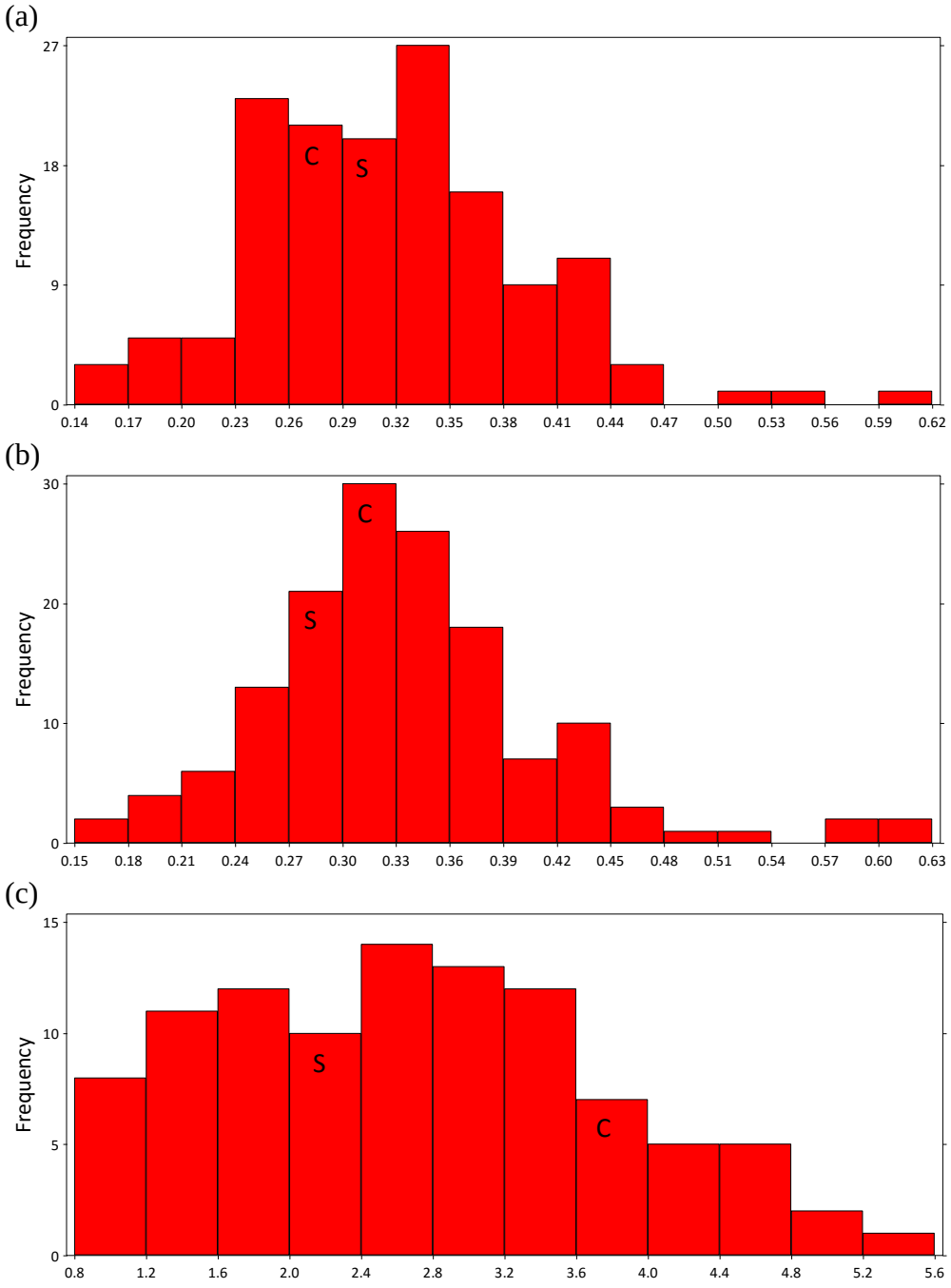
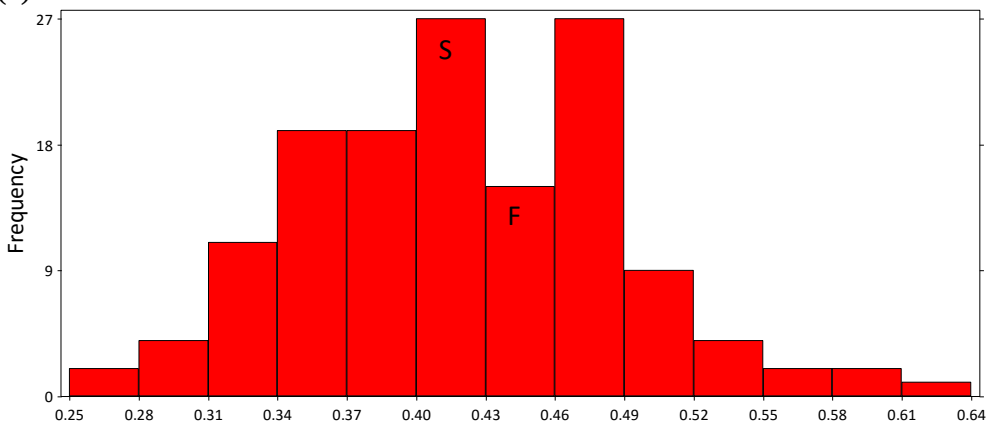
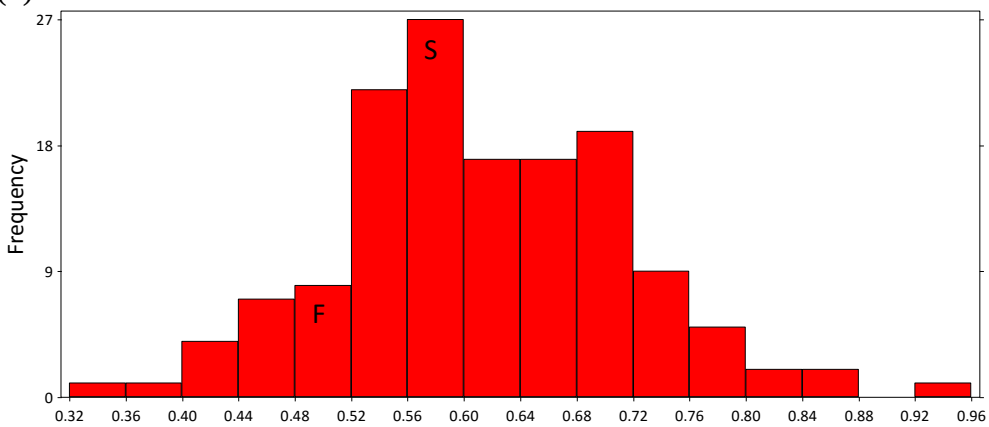


Figure 3.4: Histograms showing the frequency distribution of the SF populations for shoot biomass in grams for (a) 2014, (b) 2015, and (c) 2016 experiments. Note that all histograms are in different scales due to differences created from the various duration of each experiment. Groups in which the parents are found are marked by "S" for Sonora and "F" for Foisy.

(a)



(b)



(c)

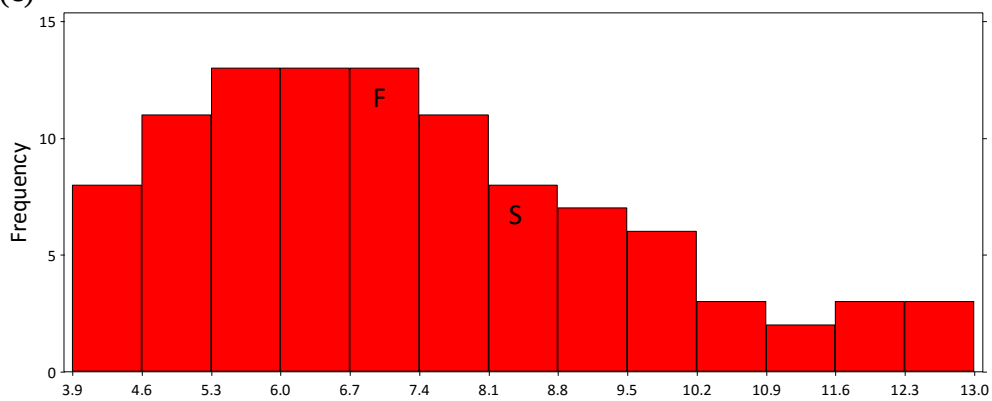
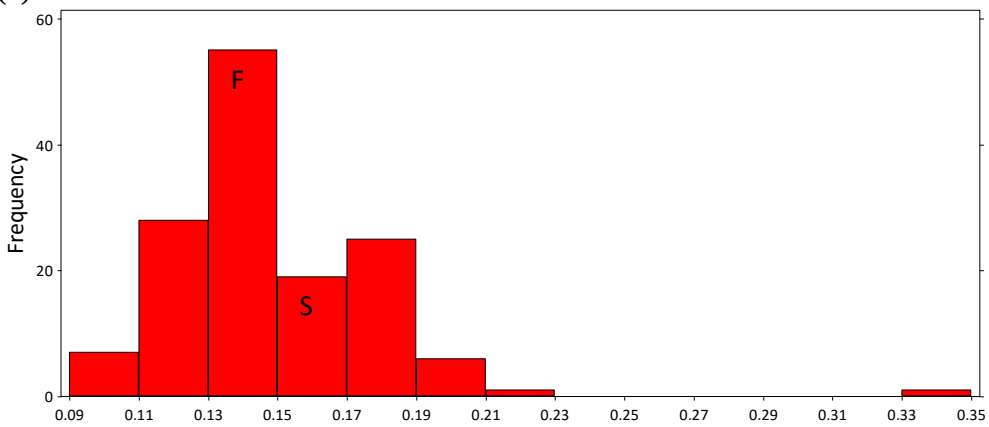
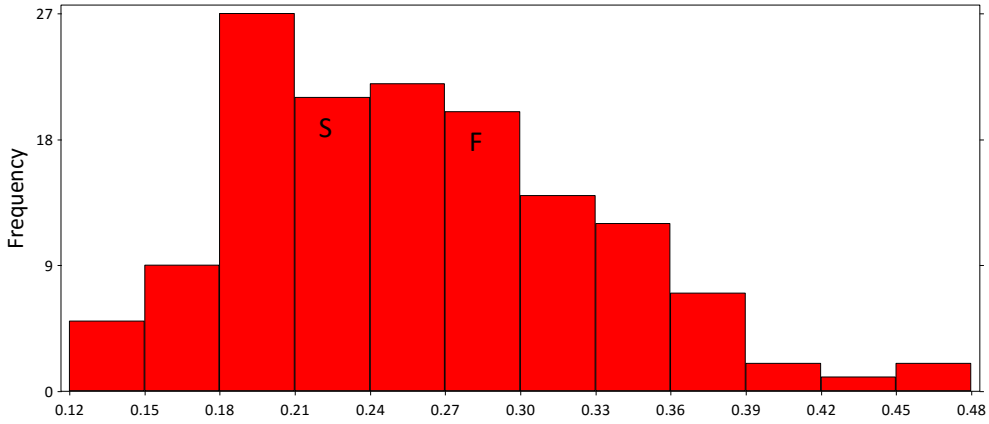


Figure 3.5: Histograms showing the frequency distribution of the SF populations for root biomass in grams for (a) 2014, (b) 2015, and (c) 2016 experiments. Note that all histograms are in different scales due to differences created from the various duration of each experiment. Groups in which the parents are found are marked by "S" for Sonora and "F" for Foisy.

(a)



(b)



(c)

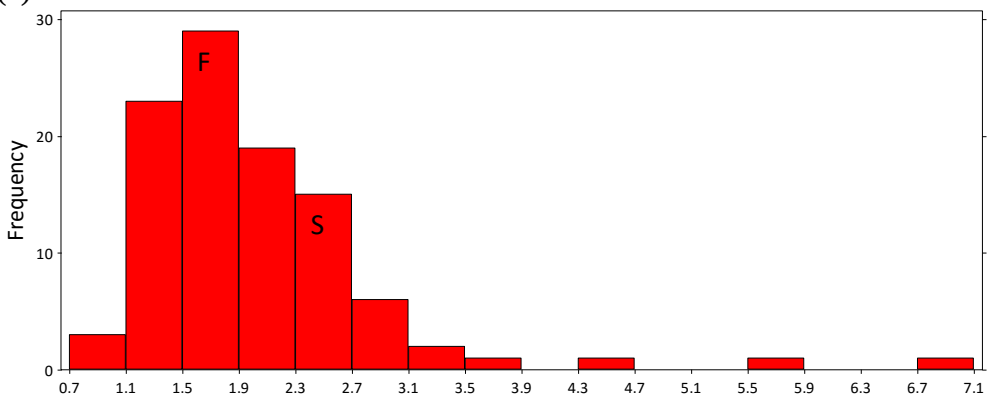
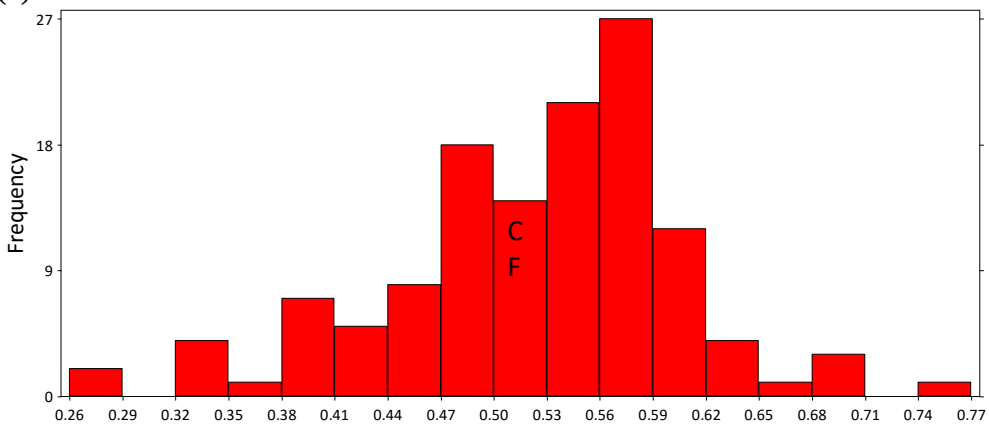
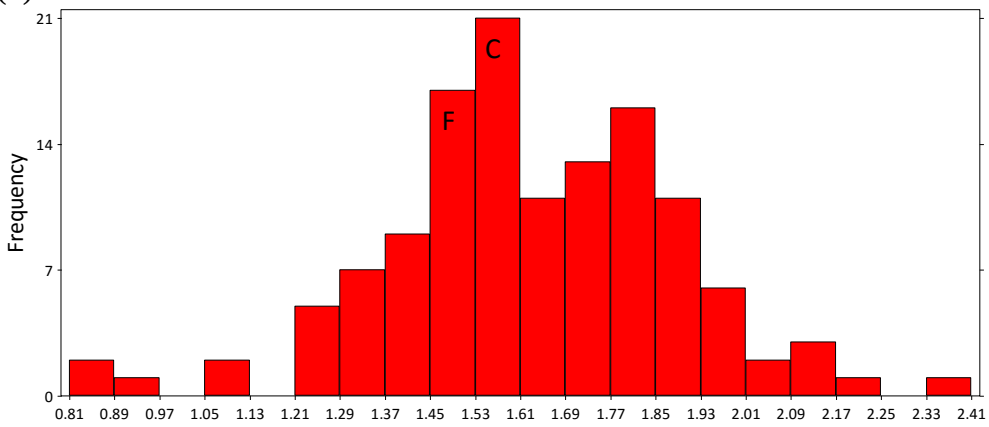


Figure 3.6: Histograms showing the frequency distribution of the CF populations for shoot biomass in grams for (a) 2014, (b) 2015, and (c) 2016 experiments. Note that all histograms are in different scales due to differences created from the various duration of each experiment. Groups in which the parents are found are marked by "C" for CBdeM and "F" for Foisy.

(a)



(b)



(c)

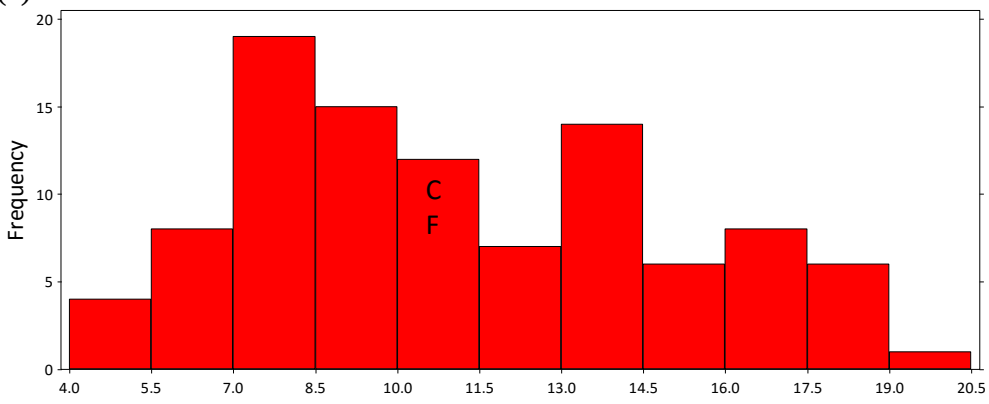
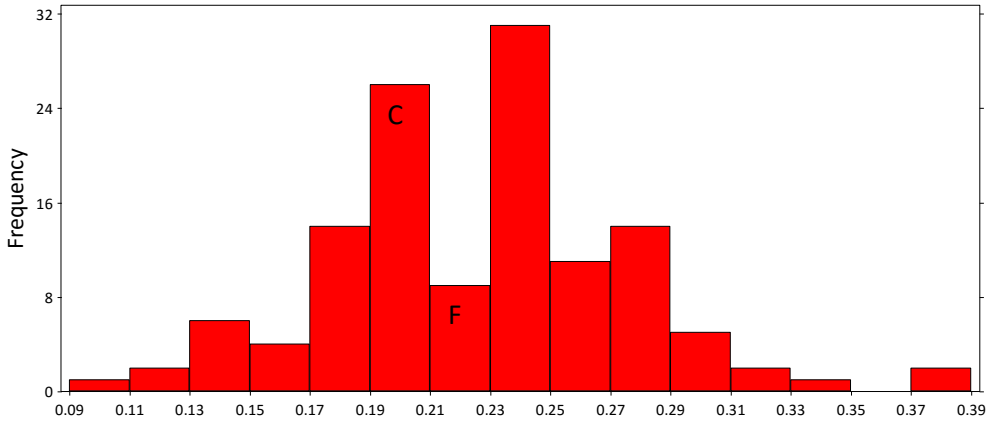
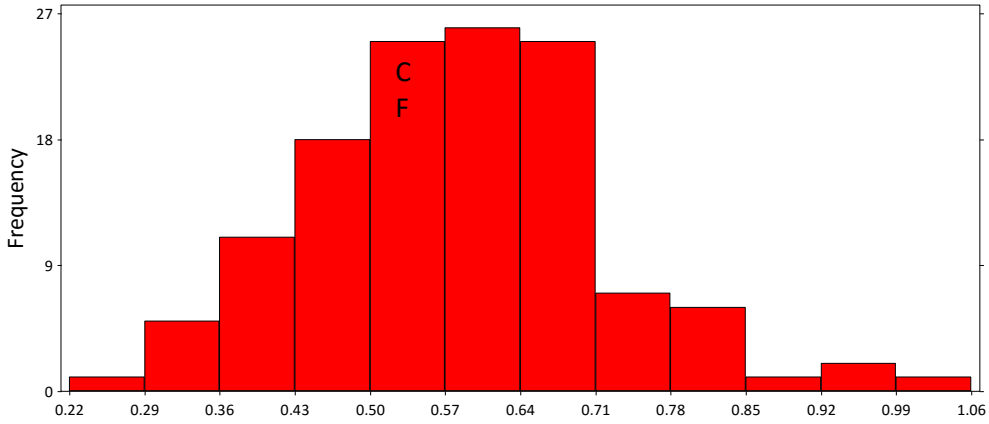


Figure 3.7: Histograms showing the frequency distribution of the CF populations for root biomass in grams for (a) 2014, (b) 2015, and (c) 2016 experiments. Note that all histograms are in different scales due to differences created from the various duration of each experiment. Groups in which the parents are found are marked by "C" for CBdeM and "F" for Foisy.

(a)



(b)



(c)

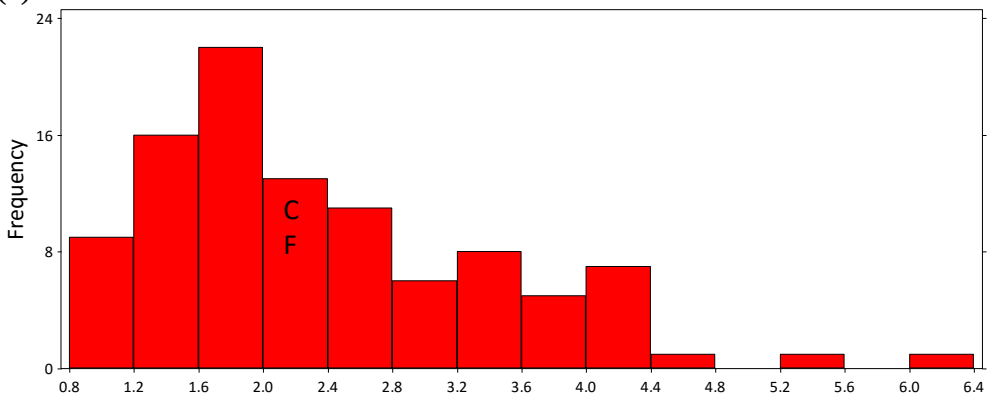


Figure 3.8: Scatter plots for the combined data of (a) the tradeoff experiments and data provided by Dr. Harun Bektas, (b) the allelic variation experiments and the 2016 mapping population experiments, and (c) the 2014 and 2015 mapping population experiments. Scatter plots show (a) shoot mass (SM) versus root mass (RM) and grain yield (GY) versus root mass (RM) and (b, c) shoot mass (SM) versus root mass (RM) as recorded in grams. Each point represents an individual plant from the various experiments which were grown until (a) maturity, (b) 40-70 days, and (c) 21-28 days. Note that all plots are in different scales (Loess $\alpha = 0.75$).

(a)

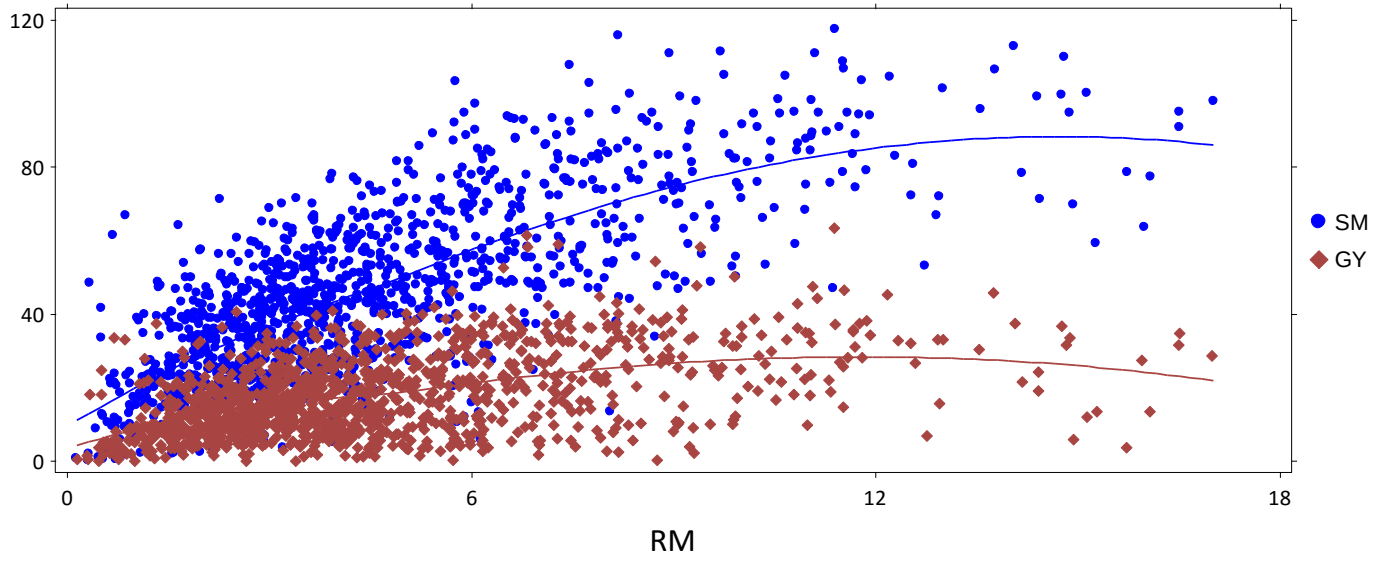
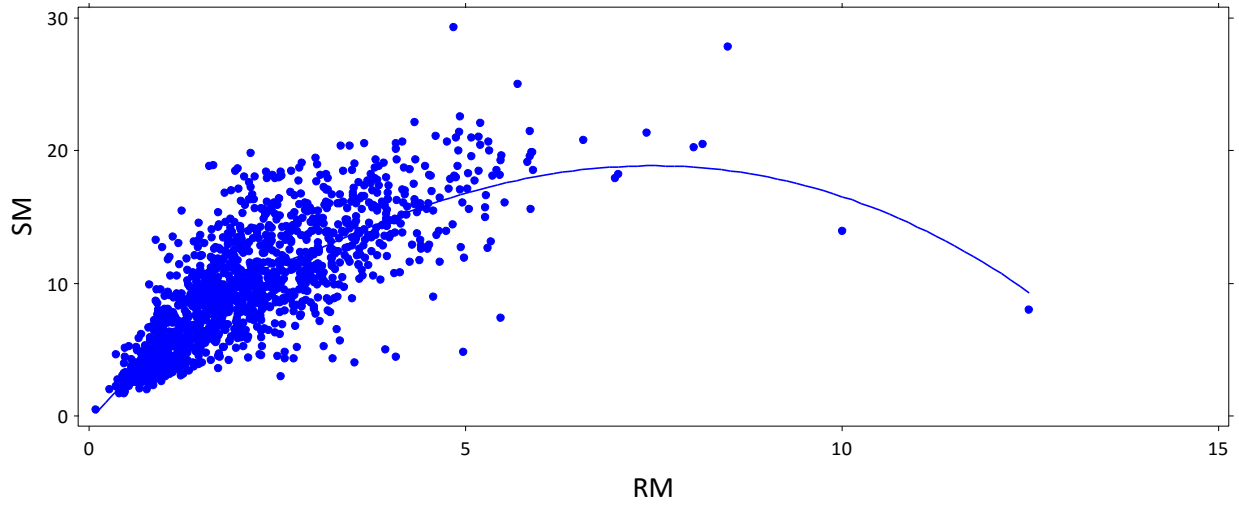


Figure 3.8 Continued.

(b)



(c)

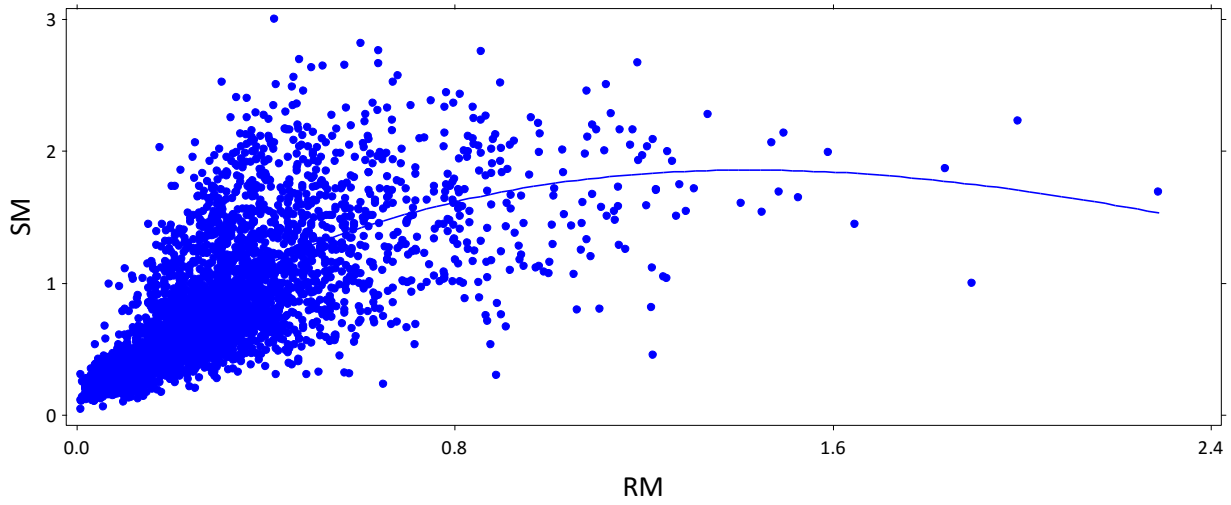
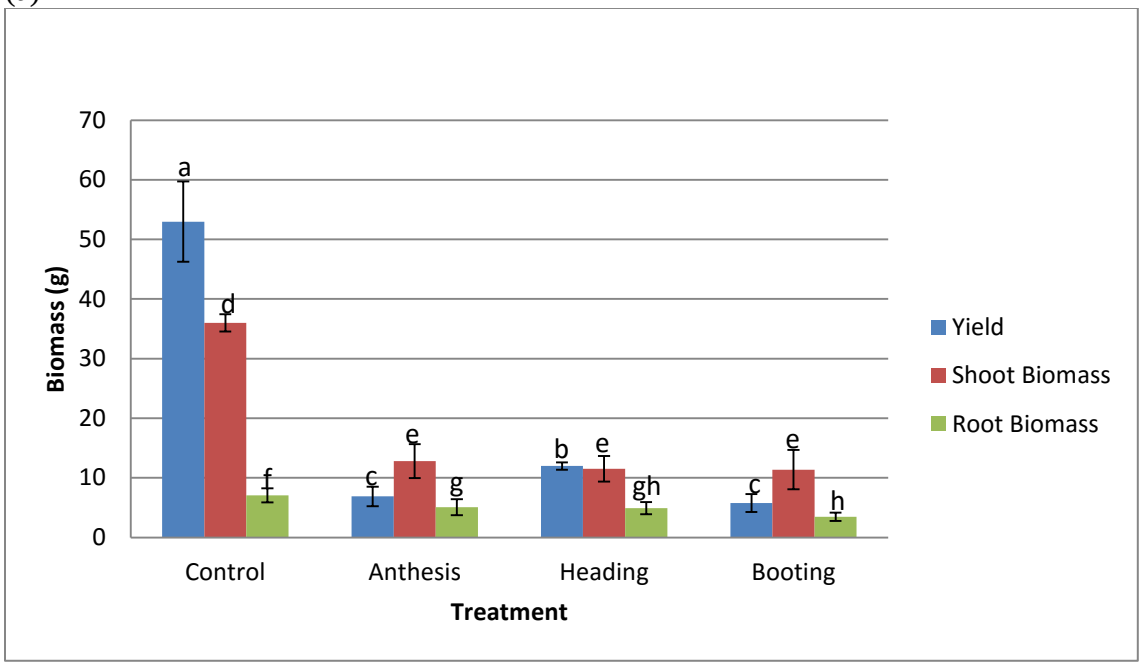


Figure 3.9: Bar graphs displaying the results for (a) Pavon 76 and (b) Yecora Rojo from the pot system used in the tradeoff experiments. Groups are designated by different letters (LSD $p<0.05$).

(a)



(b)

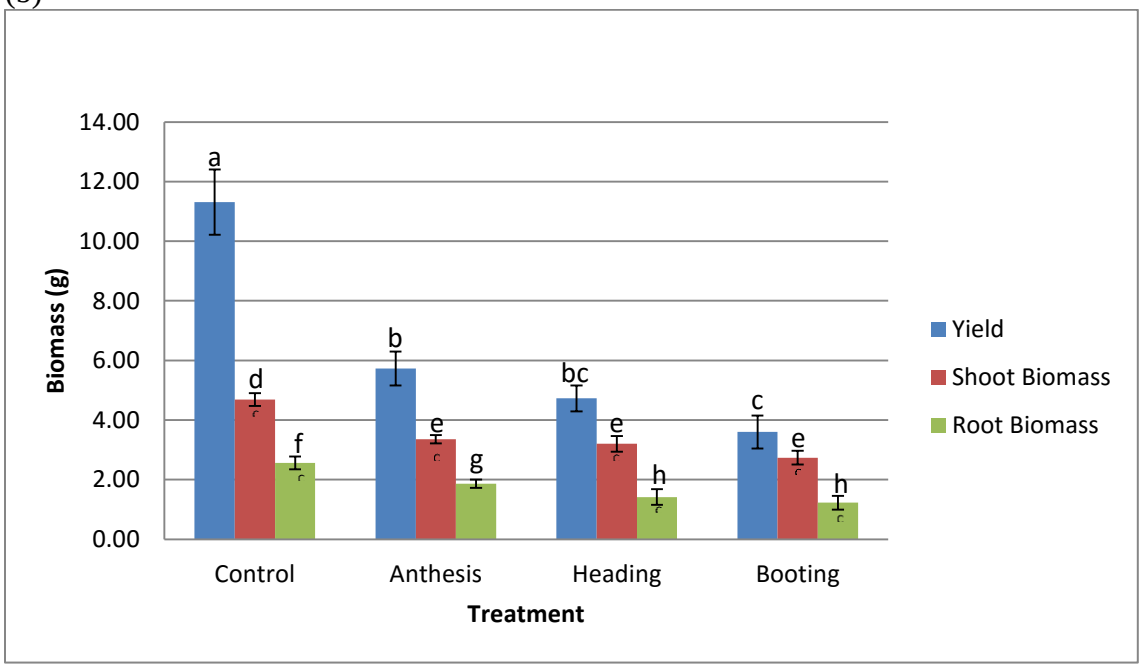
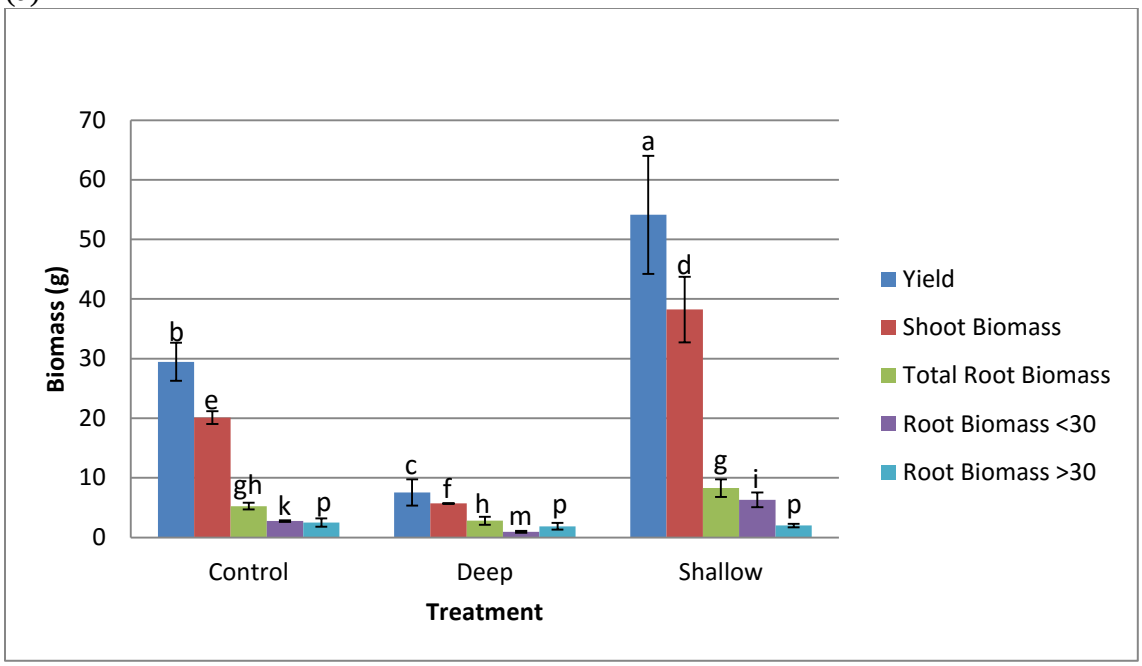


Figure 3.10: Bar graphs displaying the results for (a) Pavon 76 and (b) Yecora Rojo from the tube system used in the tradeoff experiments. Groups are designated by different letters (LSD $p<0.05$).

(a)



(b)

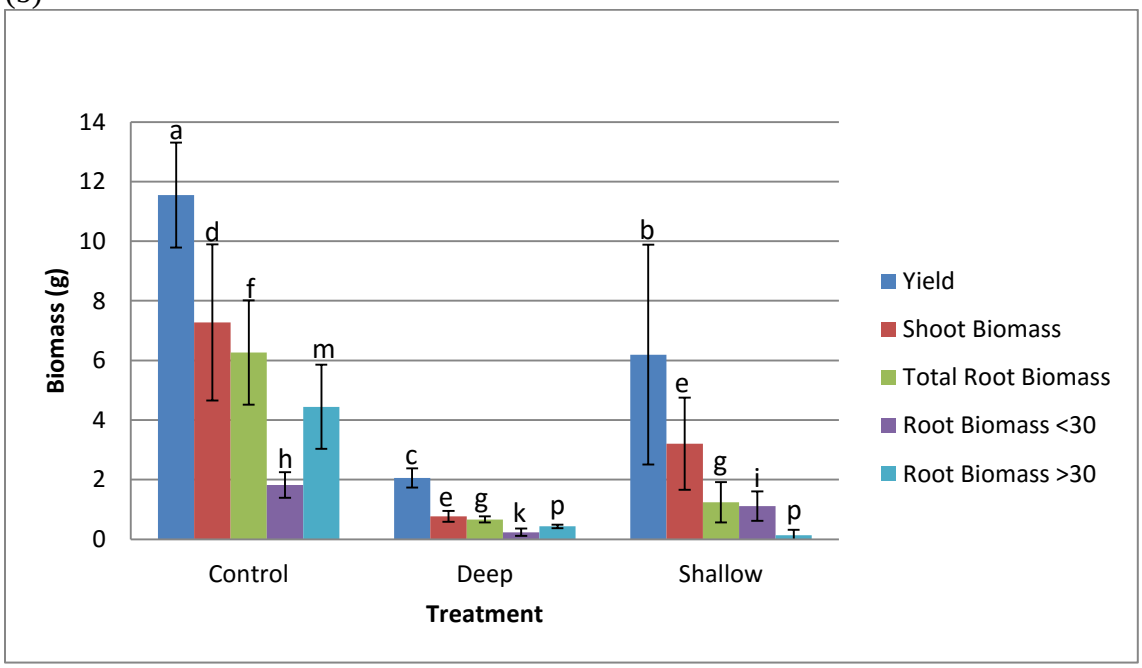
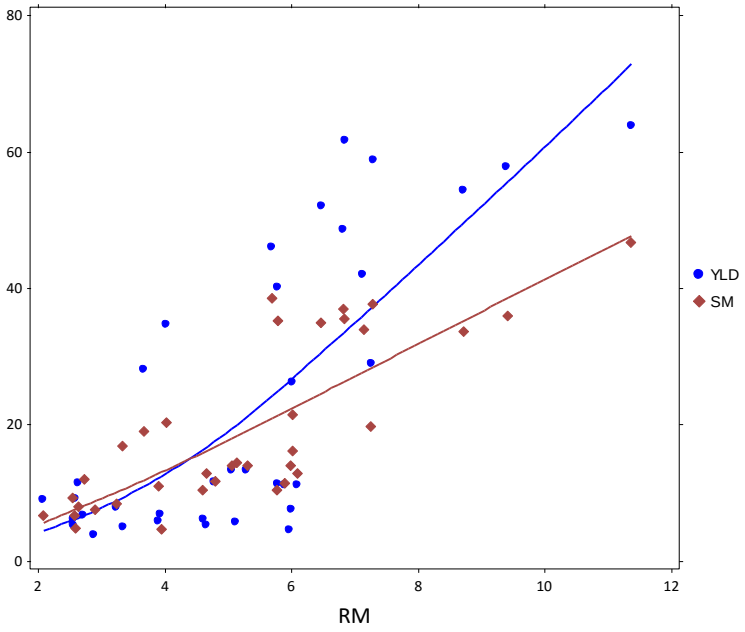
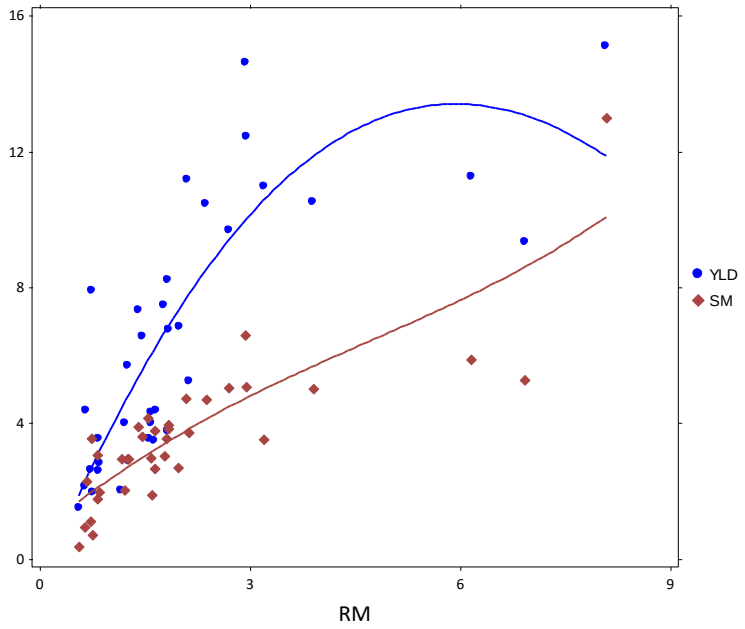


Figure 3.11: Scatter plots for root biomass (RM) in grams plotted against shoot biomass (SM) and yield (YLD) in grams for cultivars (a) Pavon and (b) Yecora Rojo. Data shown are combined from both systems used in the tradeoff experiments. Note that scatter plots are in different scales (Loess α = 2.0).

(a)



(b)



Chapter 4

Testing Near Isogenic Lines for Allelic Variation at Loci Controlling Root Biomass in Bread Wheat

Abstract

Introduction of the short arm of rye chromosome 1R (1RS) into wheat has significantly increased grain yields. Studies have shown that 1RS carries a locus controlling root biomass and improves canopy water status under water stressed conditions. A general genetic map location of the locus is known but allelic variation would further facilitate the identification of the responsible gene(s). To this end, six 1RS.1BL translocations from various sources in three different genetic backgrounds were tested for root biomass and response to drought but no significant differences among different 1RS arms were observed. Behavior of the 1RS.1BL translocation in cv. Pavon 76 in various drought experiments suggested that wheat chromosome arm 1BS of Pavon 76 possibly carries a locus for root system plasticity in response to drought. A set of 15 substitutions of chromosomes 1B from various sources, in the same genetic background of cv. Pavon 76, were tested for root biomass in various experiments. Again, no significant variation among all 1B substitution lines was observed. These results suggest that either no allelic variation at the targeted loci exists, or the sets of lines were biased. While various 1B chromosomes originated from a random sample of wheats, 1RS arms were derived from various triticales, perhaps preselecting certain allelic combinations.

Introduction

The introduction of the short arm of rye chromosome 1R (1RS) has been shown to increase root biomass in wheat (Ehdaie et al. 2003) and is most likely responsible for the remarkable popularity of the 1RS.1BL translocations in wheat breeding around the world (Rabinovitch 1998). Using wheat-rye 1BS-1RS recombinants, the region of 1RS suspected of carrying the locus leading to increased root biomass has been narrowed down (Howell 2014); however, an insufficient number of crossover points in the critical region has delayed identification of the responsible gene(s). Production of new 1BS-1RS recombinants is a very consuming proposition (Lukaszewski 2000; Anugrahwati et al. 2008). Allelic variation at the root locus on 1RS would make genetic mapping possible and perhaps accelerate progress in identifying the precise gene(s). For those reasons, a set of 1RS.1BL translocation chromosomes with 1RS arms originating from various sources (A.J. Lukaszewski, pers. comm.) were tested for their effects on root biomass.

Introduction of rye chromosome arm 1RS into wheat affects root architecture and response to drought. Similarly, introgression of a segment of chromatin from an Agropyron species affected drought tolerance in wheat (Placido et al. 2013). However, wheats themselves appear to have several mechanisms that may increase their adaptation to water stress conditions. One of those is defined as phenotypic plasticity, however, we know very little about the genetic basis of variation in quantitative traits, and even less about the genetics of plastic responses (Nicotra and Davidson 2010; Via et. al. 1995). Ehdaie et al. (2012) indicated that cv. Pavon 76 may possess considerable root system

plasticity in response to drought. In their experiments Pavon 1RS.1BL translocation lines had greater root biomass per plant as compared to Pavon 76. However, Pavon 76 responded to drought differently than its isogenic 1RS.1BL translocation lines by producing more root biomass when compared to the well-watered conditions. This led to the conclusion that a gene, or genes, affecting adaptive phenotypic plasticity of the root system may be located on chromosome arm 1BS. Since there are wheats that do not exhibit the same plastic response as Pavon 76, chances are that there may exist allelic variation for genetic factors for root plasticity on 1BS. As a set of single chromosome substitutions lines of 1B from varied sources already existed (A.J. Lukaszewski, pers. comm.), these stocks were tested under uniform conditions.

Materials and methods

Two systems were used to tests the plant materials. The first consisted of 10.16 cm x 80 cm PVC tubes fitted with a plastic sleeve filled with 5.6 kg of sand. The plastic sleeve had two holes at the bottom covered with filter paper to allow for drainage and retain the sand. This system is the same as that used by Ehdaie and Waines (2006). Experiments were setup in a factorial design with four replications that were treated as blocks. Tubes were brought to water holding capacity and seeds were imbibed for 24 hours before planting. All tubes received the same amount of water per day until booting at which point the drought treatment received 60% less water each day until the experiments were terminated. The second system consisted of one gallon pots setup in a

randomized complete block design with 8 replications treated as blocks. Pots were lined with a plastic sleeve, filled with 3 kg of sand and four holes were punctured in the plastic for drainage. Pots were brought above water holding capacity and allowed to drain for 24 hours before being planted. Seeds were imbibed for 24 hours and then planted into the sand filled pots with one seed per pot. Pots were maintained at water holding capacity for the duration of the experiments.

In both systems after the experiments were terminated plants were processed in the same manner. Shoots were separated from the roots by harvesting them at the sand level. Roots were then washed clean of sand. Shoots and roots were dried for 72 hours in a forced air drier at 80°c at which point they were weighed for total biomass.

Six 1RS.1BL translocation lines were tested in the three uniform genetic backgrounds of cv. Pavon 76, cv. Hahn, and breeding line UC1110 (kindly provided by Dr. J. Dubcovsky, University of California, Davis). The 1RS arms originated from chromosomes 1R transferred to Pavon 76 from various sources: the original translocation 1RS.1BL from Aurora/Kavkaz, here taken from cv. Genaro (abbreviated, 1RSv), another 1RS.1BL translocation reportedly created at CIMMYT (1RScim) and new translocations generated from chromosomes 1R taken from triticales Anoas (1RSan), Salvo (1RSsa), PI386148 (1RSmt), and a wheat line E12165 from CIMMYT with 1R(1D) substitution (1RSe). Of the six, five 1RS arms originated from *Secale cereale* and one, from PI386148, appears to be from *S. montanum*. All lines had seven backcrosses to Pavon 76 and three backcrosses to Hahn and UC1110. In Pavon 76 1RSv, 1RSan, 1RSsa, 1RSe, and 1RSmt were tested and grown until anthesis in spring 2012 and for 40 days in fall

2012 using the tube system. In Hahn 1RScim, 1RSsa, 1RSe, and 1RSmt were tested and 1RScim, 1RSan, 1RSsa, 1RSe, and 1RSmt were tested in UC1110. Plants were grown for 50 days in winter and spring of 2015 in the pot system.

Fifteen substitution lines of chromosome 1B from various spring and winter wheats were developed in the background of cv. Pavon 76 (A.J. Lukaszewski, pers. comm), for a different project, however, they are well suited for the purposes of these experiments given the diversity of the sources. All backcrosses were made to the Pavon Dt.1BL stock with seven backcrosses completed. Of these, nine were tested in spring 2013 and all fifteen were tested in fall 2013. Chromosome 1Bs originated from an Iranian landrace #55 (abbreviated, 1B55), cvs. Begra (1Bbe), Broma (1Bbr), Cheyenne (1Bcn), Chinese Spring (1Bcs), Culver (1Bne), Glenlea (1Bgl), Henika (1Bhe), Little Club (1Blc), Luna (1Bln), Selkirk (1Bse), Tambor (1Bta), breeding line KOC 299 (1Bko), Thatcher (1Bth), and Wheaton (1Bwh). Pavon 76 was included as a control in both experiments and Pavon Dt.1BL was included in fall 2013. Pavon Dt.1BL is identical to Pavon 76 except it is missing the short arm of chromosome 1B. If the plasticity locus is on 1BS Pavon Dt.1BL should not show any plastic response. Plants were grown until anthesis in spring 2013 and for 40 days in fall 2013 in the tube system.

Results

The 1RS.1BL translocation lines showed no significant differences in the genetic background of Pavon 76 or Hahn (Figure 4.1 and 4.2). For Pavon 76 standard deviations

ranged from 0.91 to 6.21 in spring 2012 and from 0.14 to 0.96 in fall 2012 for shoot biomass. For root biomass standard deviations ranged from 0.17 to 1.81 in spring 2012 and from 0.09 to 0.54 in fall 2012. Standard deviations in Hahn ranged from 1.81 to 2.67 in winter 2015 and from 0.79 to 3.27 in spring 2015 for shoot biomass. Root biomass standard deviations ranged from 0.37 to 1.19 in winter 2015 and 0.20 to 0.52 in spring 2015. In UC1110 the 1RSsa.1BL translocation line had significantly larger root biomass than all others, including UC1110 (Figure 4.3). Standard deviations for shoot biomass ranged from 2.12 to 2.74 in winter 2015 and from 2.08 to 5.09 in spring 2015. Root biomass standard deviations ranged from 0.59 to 1.04 in winter 2015 and from 0.27 to 0.82 in spring 2015. In the winter 2015 experiments 1RSsa.1BL had an average root biomass of 4.96 grams while all others had an average of 3.31 grams per plant. In the spring 2015 experiments 1RSsa.1BL had an average root biomass of 3.09 grams and all others had an average of 1.98 grams per plant.

Pavon 1B substitution lines demonstrated no significant differences between one another or when compared to Pavon 76 and Pavon Dt.1BL (Figure 4.4). Standard deviations for shoot biomass ranged from 0.68 to 6.21in spring 2012 and from 0.21 to 1.80 in fall 2012. Root biomass had standard deviations ranging from 0.16 to 1.77 in spring 2012 and from 0.12 to 3.28 in fall 2012.

Discussion

Identification of specific genetic loci contributing to the differences observed in wheat root systems and their relationship with shoot biomass and grain yield would be an important step in all attempts at manipulation of these characteristics. Understanding the genetic basis of root biomass will enable further understanding of how this trait influences yields under drought.

It is known that there is a locus on 1RS originating from the Aurora/Kavkaz source that contributes to improved yields and canopy water status under water stress (Howell et al 2014) and it appears to be the same locus as the one contributing to increased root biomass (Sharma et al. 2011). It is likely that this locus is responsible for continuing interest in the 1RS.1BL translocation in wheat.

The sets of lines in cvs. Pavon 76 and Hahn with 1RS chromosome arms originating from various sources showed no apparent differences for their root characteristics. Hahn naturally carries the 1RS.1BL translocation from the Aurora/Kavkaz source; unfortunately a line of Hahn without its original 1RS.1BL translocation was not available as a control when the tests were made. Perhaps there is no allelic variation in rye for this specific locus or the sample of 1RS arms tested was biased. All 1RS chromosome arms originated from hexaploid triticale, either directly, as those from Anoas, Salvo, and PI386148, which were crossed and backcrossed to Pavon 76 as complete chromosomes 1R and then translocated to 1BL, or indirectly, such as 1RSe and 1RSv, which were transferred to Pavon 76 from other wheats, obtained from triticale x

wheat crosses (A.J. Lukaszewski, pers. comm). Since triticales are bred for more demanding stands than wheat it cannot be entirely discounted that selection favors rye alleles which increase adaptation to stress. After all, the original translocation 1RS.1BL also originated from hexaploid triticale (Zeller and Hsam 1983).

Despite previous findings there were no changes in root biomass of various 1RS.1BL translocation lines relative to Pavon 76 or Hahn. For Pavon 1RS.1BL translocation lines this is rather surprising since previous publications reported increased root biomass. Our findings are understandable with regards to Hahn because it is already a 1RS.1BL translocation wheat. Within UC1110 an increase in root biomass for 1RS.1BL translocation lines was expected relative to UC1110, however, all translocations lines but one were not significantly different. Possibly these results are due to the differences of the experimental system used to test the Hahn and UC1110 1RS.1BL lines as compared to those in previous research. It is possible that the pot system constrained the roots from growing to their full potential due to limited space or the ease of access to water promoted these results. However, the Pavon 1RS.1BL lines in our study were tested using the same system as previously reported leaving no apparent explanation for the difference in results.

It is an interesting observation that one of the translocations tested, 1RSsa.1BL, where 1RS originated from triticale Salvo, showed significantly increased root biomass in the genetic background of a breeding line from UC Davis, UC1110. Although the p-value is highly significant ($p<0.01$) the biological significance may be less so. UC1110 has a standard karyotype, that is, it is disomic for normal 1B. One would expect that

introduction of 1RS from the Aurora/Kavkaz source would have a clear and measurable effect, as it does in many other wheats, but it did not. Therefore, the result for the 1RSsa arm may only be a statistical aberration, but further tests are in order.

After dozens of experiments it is apparent that cv. Pavon 76 has some unique ability to maintain root biomass under drought conditions. This can possibly be plasticity as proposed by Ehdaie et al. (2012). Several tests of root characteristics on the effects of a rather wide set of substitution of chromosomes 1B into Pavon 76 did not reveal any apparent and statistically significant differences among the lines. Given that backcrosses were made to the Dt.1BL stock; the shorts arms and approximately the proximal halves of the long arms originated, unchanged by crossing over, from the donor cultivars; the distal one half of each long arm is likely recombined with 1BL of Pavon 76. So, while it is clear that Pavon 76 does have a unique ability to respond to changes in the environment, no major genetic factor controlling it appears to be located on chromosome arm 1BS as originally proposed (Ehdaie et al. 2012). However, large variation in all studies for root biomass is common, so perhaps some subtle differences could not be detected here.

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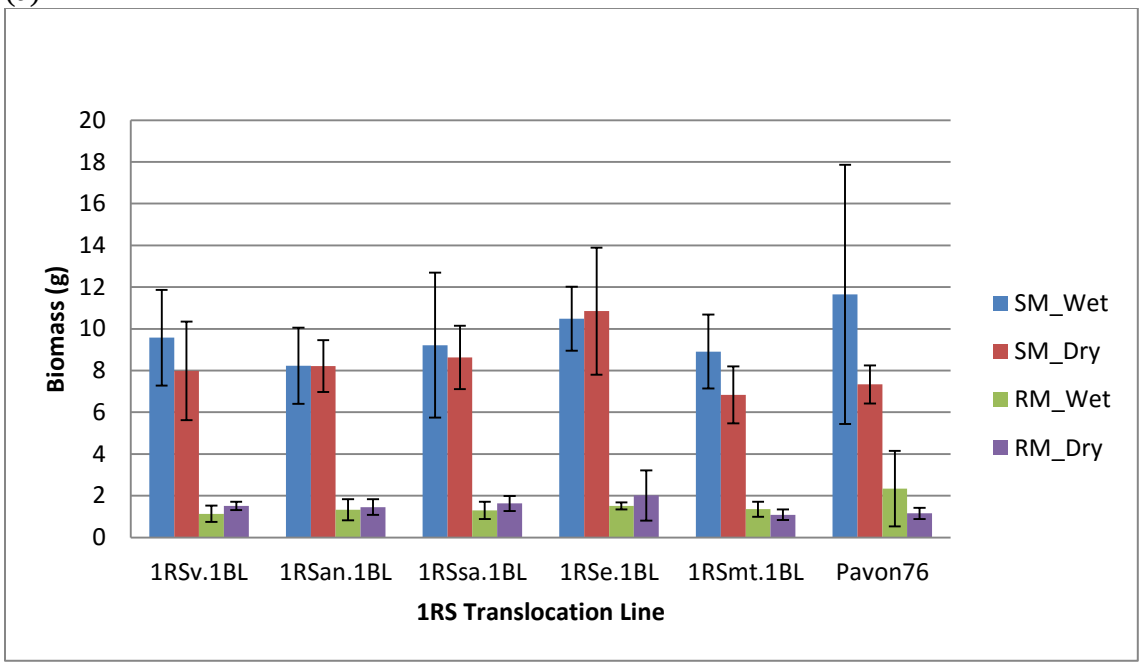
Figure 4.1: Bar graphs displaying the results for Pavon 76 and the 1RS translocation lines in (a) spring 2012 and (b) fall 2012 from the tube system used in the allelic variation experiments. All lines grouped together (LSD $p<0.05$).

(a)



(b)

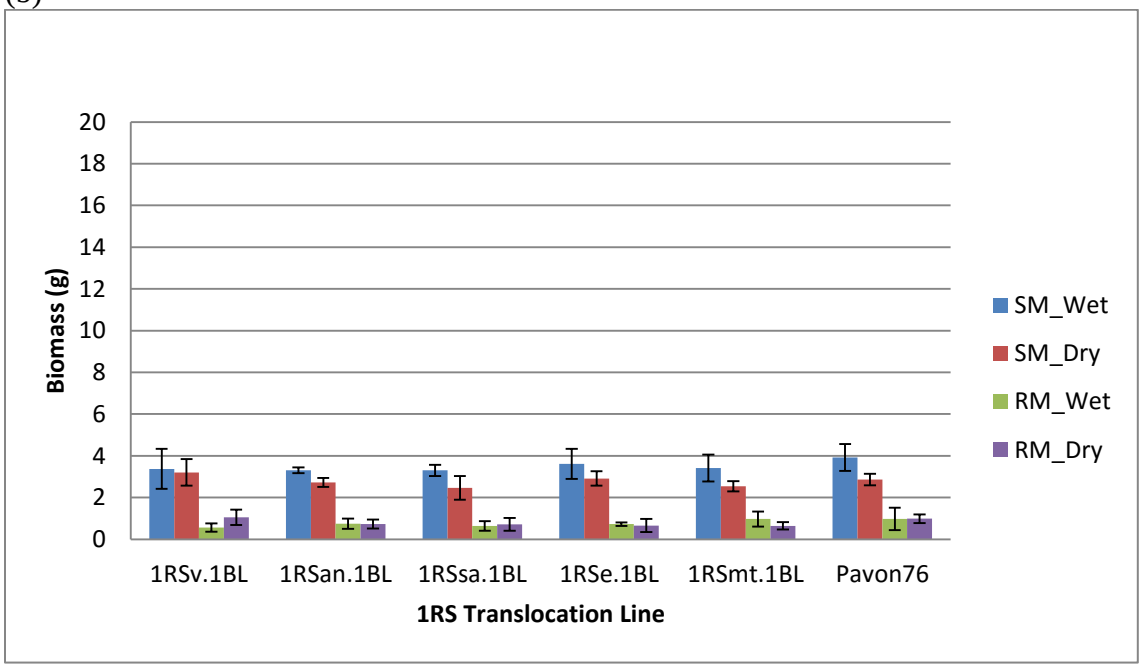
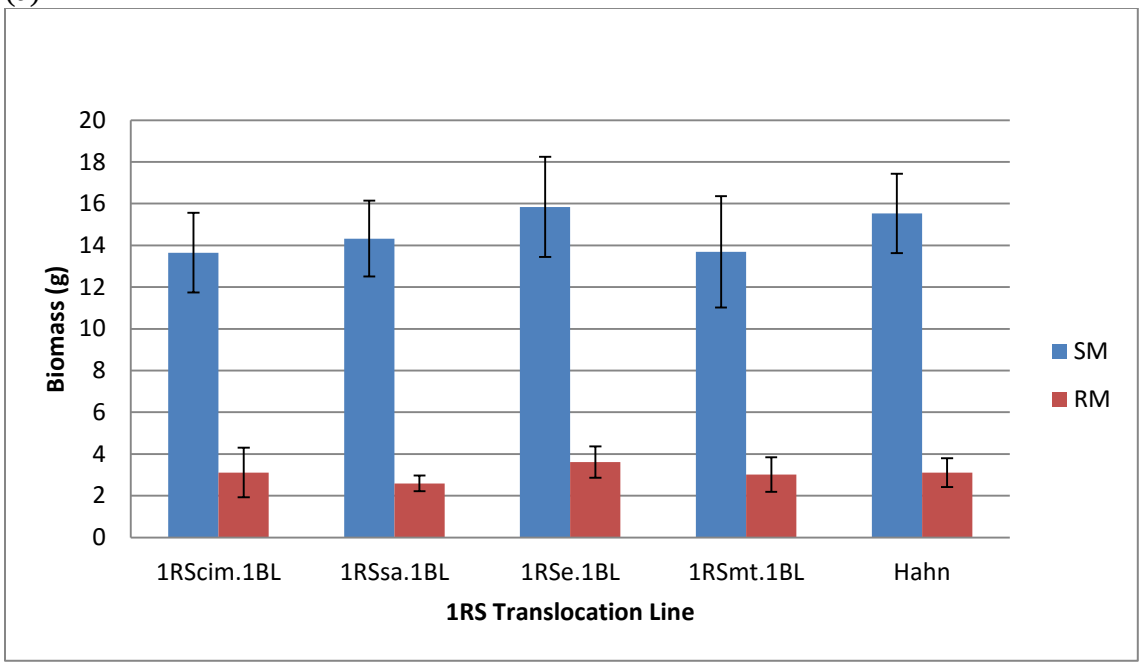


Figure 4.2: Bar graphs displaying the results for Hahn and the 1RS translocation lines in (a) winter 2015 and (b) spring 2015 from the pot system used in the allelic variation experiments. All lines grouped together (LSD $p<0.05$).

(a)



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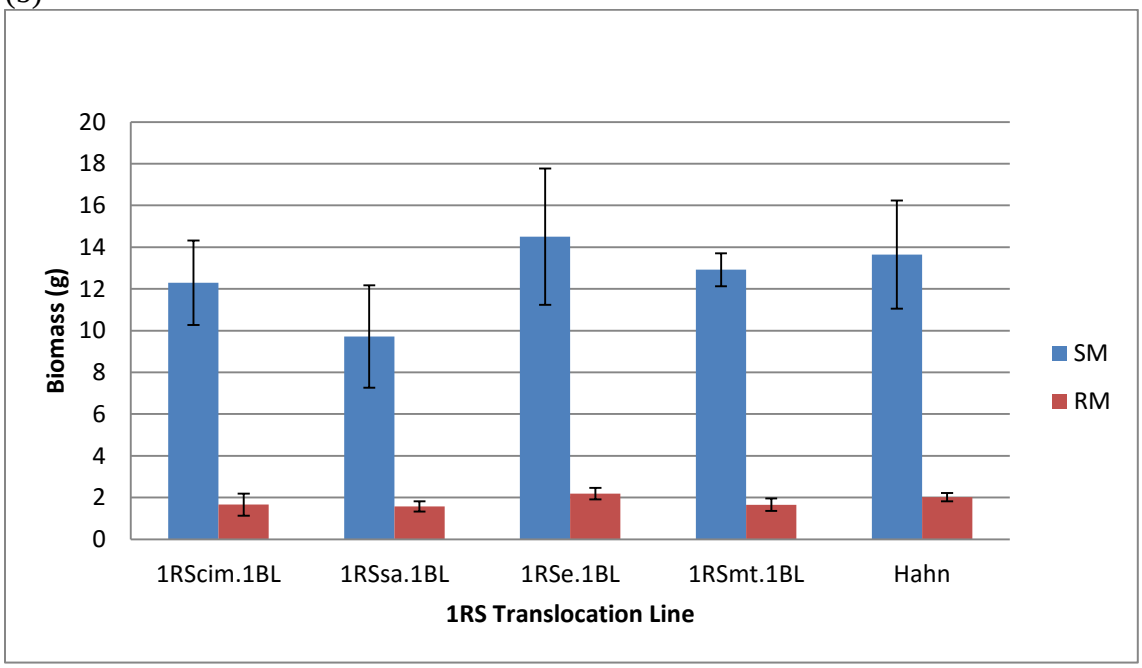
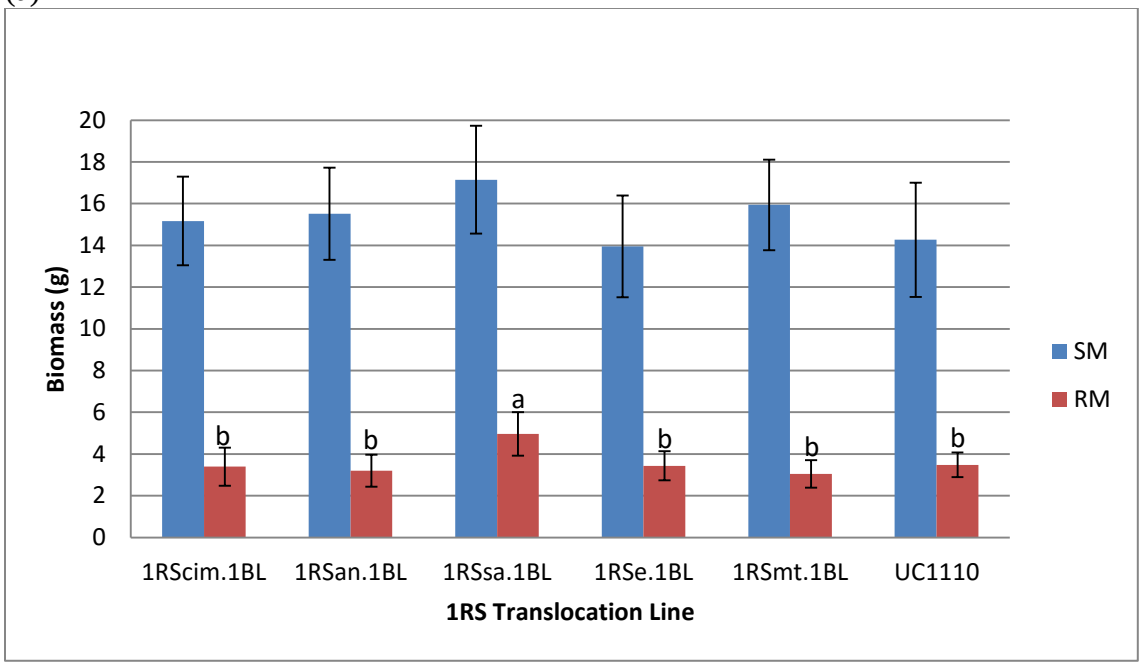


Figure 4.3: Bar graphs displaying the results for UC1110 and the 1RS translocation lines in (a) winter 2015 and (b) spring 2015 from the pot system used in the allelic variation experiments. Groups are designated with different letters (LSD $p<0.05$)

(a)



(b)

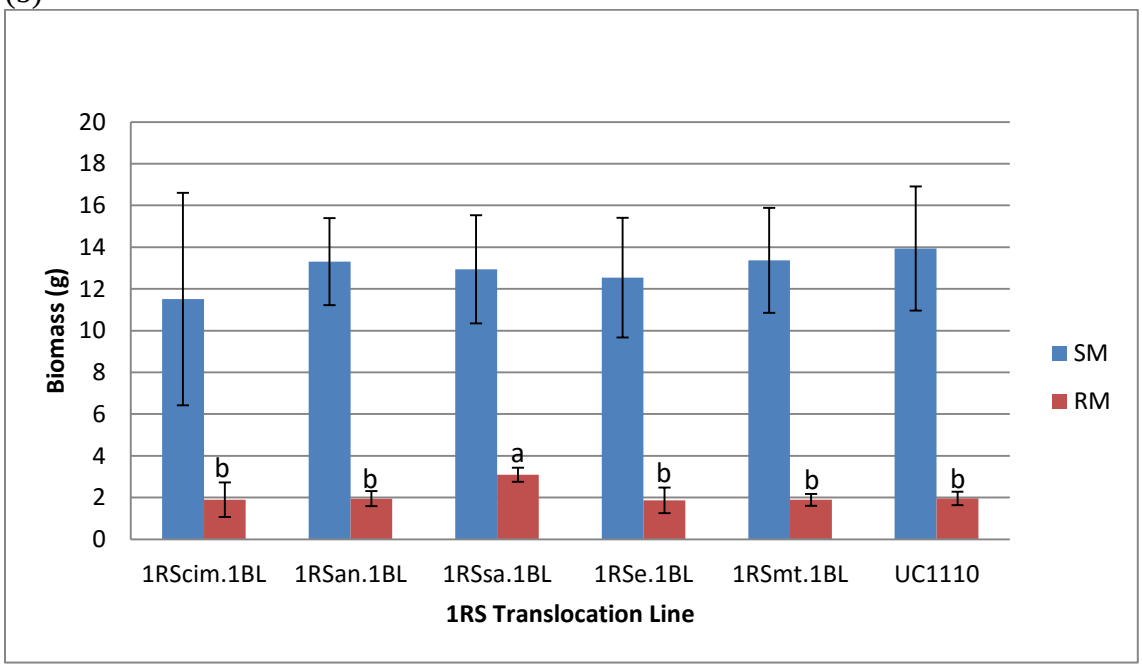
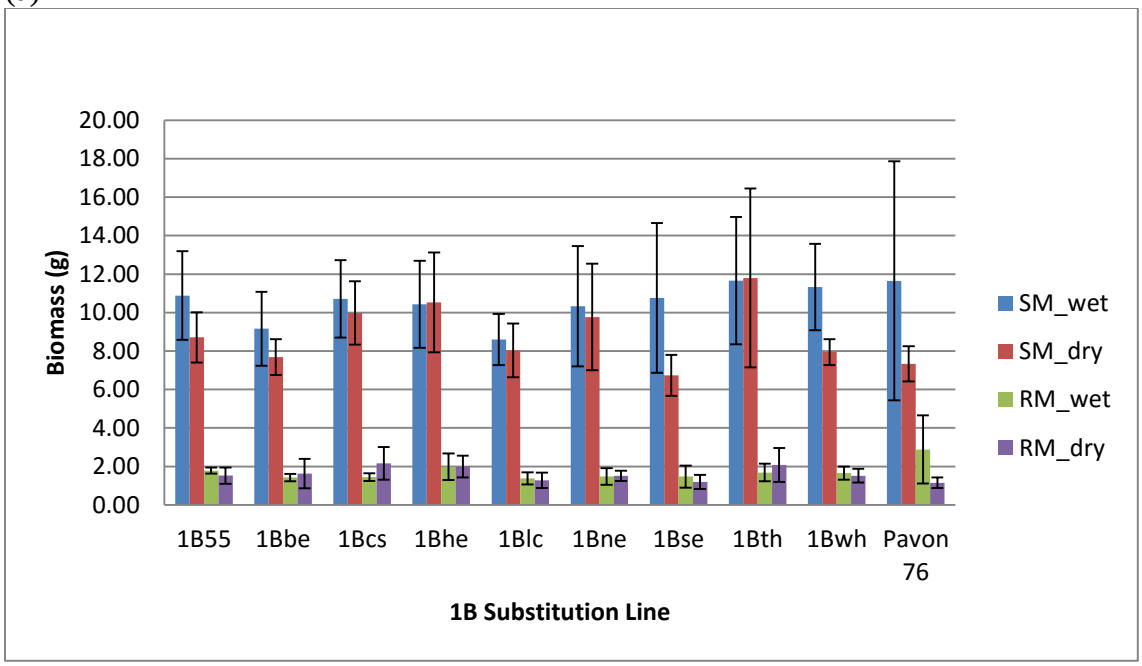
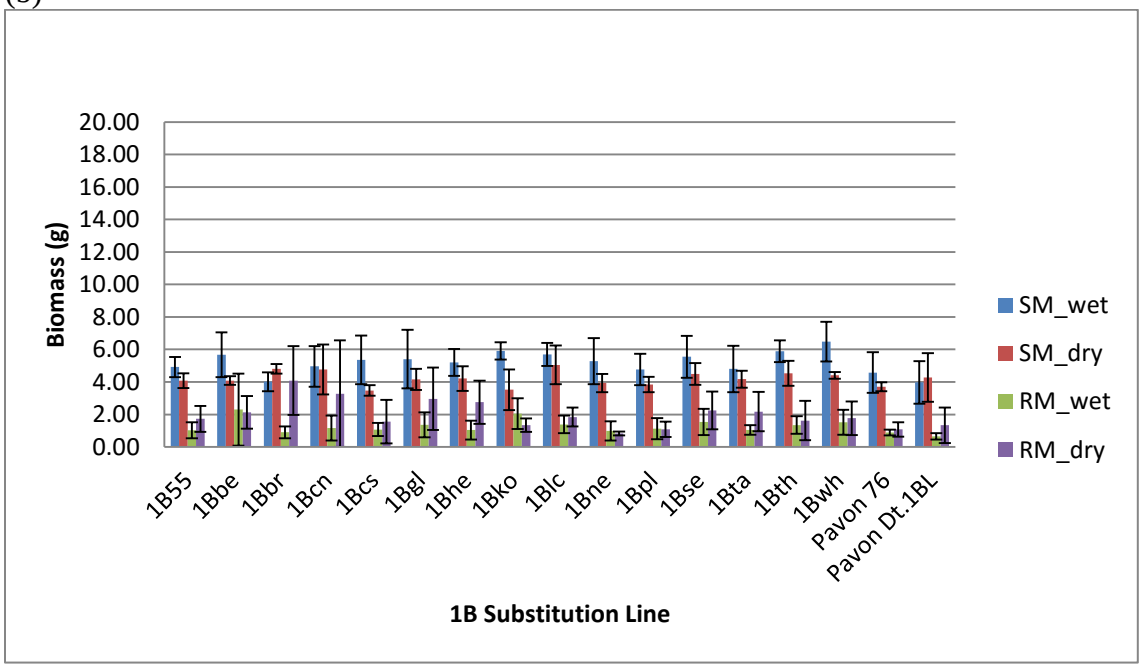


Figure 4.4: Bar graphs displaying the results for Pavon 76 and the 1B substitution lines in (a) spring 2012 and (b) fall 2012 from the tube system used in the allelic variation experiments. All lines grouped together (LSD $p<0.05$).

(a)



(b)



General Conclusions

Wheat is ranked as the number one crop in terms of production area with yields reaching ca. 700 million metric tons per year and provides approximately 20% of our daily caloric intake (FAOSTAT 2014). With the increasing rise in global food demand and increasing unpredictability of weather patterns resulting from climate change the development of new wheat cultivars that are favorably responsive to drought are greatly needed. These new cultivars not only need to maintain the current quality standards but they must also remain productive under a range of environments and stressful conditions.

In an effort towards these goals three integrated mapping populations of bread wheat were developed by crossing three spring wheats. Crosses were made in a manner so that each parent was in two of the three populations: Sonora x CBdeM (abbreviated as SC), Sonora x Foisy (SF), and CBdeM x Foisy (CF). Initially those populations consisted of ca. 238 lines on average; however, after screening and subsequent genotyping they have an average of ca. 138 lines. Since each parent in in two of the three populations this gives an average population size of ca. 276 lines and a system for instant verification of QTL across populations. High density genetic maps for each population were also generated with an overall average of ca. 51.6 markers per linkage group and average marker spacing of ca. 3.76 cM. The quality of these maps was tested by comparison to the consensus map of Wang et al. (2014) and mapping of some well-known agronomic traits. For awn type two awn inhibitor loci, *B1* and *B2*, were identified as coming from the parents Foisy and Sonora, respectively. The QTL for *B1* on chromosome arm 5AL

was further verified by the previous mapping of Mackay et al. (2014). For days to heading multiple QTLs were mapped and QTLs for the *Ppd-D1* locus in two of the three populations were identified with the parents Sonora and CBdeM as the responsible QTL donors. Additionally, a QTL was located on chromosome arm 5BL that was previously mapped by Zanke et al. (2014) and determined to be an *Hd6* related gene having a major impact on heading time in wheat. Finally, two QTLs for hybrid necrosis were detected on chromosome arms 5BL and 2BS which are likely to be the Ne_1 and Ne_2 loci (Tsunewaki 1970, Zeven 1972, Nishikawa et al. 1974). With those simple mapping exercises the quality and accuracy of the linkage maps developed for these populations was able to be verified. Using these data as an example it was demonstrated that the three populations provide a great tool for the instant verification of QTLs across populations. This demonstrates that these populations should become a valuable tool for further research on more complex traits like root architecture and drought tolerance.

With the linkage map quality verified, the three populations were utilized to investigate QTLs for seminal root traits. Both seminal root angle and number were found to have high heritability in the populations. All populations showed significant variation for both traits as well over the two years that experiments were conducted. In total 31 genomic regions were associated with seminal root angle and number. Of those regions, three QTLs for seminal root angle were consistent from one year to the next on chromosome arms 2DS, 6AL, and 7BS within populations and one of those (2DS) was verified across two of the three populations. For seminal root number only one QTL was consistent in the SF population on chromosome arm 4BL with no QTL being verified

across populations. When compared to other studies on these two traits the results are similar in that QTLs are rarely consistent across populations and highly variable. Part of these traits complexity may be explained by the interactions that they have with one another as well as seed weight. In the three populations the correlations of these three traits show different relationships. In the SC population root angle and number were negatively correlated. The SF population showed that seminal root number and seed weight were positively correlated which has been observed by other research as well (Robertson et al. 1979, Christopher et al. 2013). However, the most interesting results came from the CF population which showed that seminal root angle, number, and seed weight were all correlated and explained all variation seen within the population. Upon further investigation it turned out that QTLs for seminal root angle and number were actually QTLs for seed weight. These relationships lead to more questions than answers though, in that there are no good ideas as to why the correlations are different in the three populations. What we are left with are some insights into issues that should be considered when planning future research on the matter and new dimensions to be explored. Previous studies on root characteristics in wheat have identified numerous regions associated with these traits and most have not considered these interactions or mapped seed weight as well. Those presumed QTLs need to be verified across years and in other populations before any conclusions can be drawn on the topic and it seems as though there is a long road ahead before the traits are untangled. Overall, seminal root angle is proving to be less simple than originally proposed and the story will be much more interesting as well.

Seminal root traits are not likely to be the only important factors of root architecture that will contribute to improved yields under drought. Root biomass is another trait that has researcher's interest for good reason. It is known that plants with greater root biomass and deeper roots are able to better explore the soil for available water. This gives them an advantage when water and nutrients are a limiting factor. Increased root biomass has been shown to increase grain yield under limited or rain-fed environments (Palta et al. 2011). What is not well-known is how the relationship of roots and shoots may effect plant performance and yield. Using a set of unique experiments those relationships were investigated and QTLs for shoot and root biomass were mapped. Additionally, two sets of unique cytological stocks were used to investigate allelic variation for a locus controlling root biomass on chromosome 1RS and a proposed locus on chromosome arm 1BS of wheat.

The three mapping populations showed significant variation for root and shoot biomass. However, no QTLs were consistent over multiple years or across populations. This was attributed to the large variation for biomass observed within genotypes that inflated the critical values for comparison between genotypes. A general trend was observed where the broad sense heritability for shoot and root biomass increased as the duration of the experiments increased. Shoot and root biomass were also shown to be positively correlated and their correlation became stronger as the duration of the experiments increased as well. In 2016 heading date explained 88.8 and 78.1 % of the variation seen for shoot and root biomass across the three populations. From those results it is clear that shoot and root biomass are directly related to heading date for these

populations. These findings were further supported by shoot and root QTLs detected which fall into the same region as heading time QTLs.

In general, when combining over ca. 6,000 data points, it was observed that shoot and root biomass increase proportionately until a certain threshold, however, as root biomass continues to increase shoot biomass begins to decrease. This same trend was observed when root biomass was plotted against grain yield in the same set of data. When utilizing two wheat cultivars, Pavon 76 and Yecora Rojo, it was demonstrated that individual cultivars may deviate from the general trend in a substantial way. Yecora Rojo followed the general trend while Pavon 76 continued to have increased yields as root biomass increased.

In the tests for allelic variation no solid conclusions were able to be made. Of the 1RS.1BL translocation lines tested in cultivars Pavon 76 and Hahn, none showed any promise of allelic variation. One translocation line, 1RSsa.1BL, showed a significant increase in root biomass in the UC1110 background; however, further tests would be prudent to verify this result. Of the fifteen 1B substitution lines tested in Pavon 76, none were significantly different from one another. Interesting though, is the ability of Pavon 76 to maintain a relatively similar root biomass under stressed conditions. This attribute could be the phenotypic plasticity pointed out by Ehdaie et al. (2012), however, from this research the locus controlling that trait was not verified as being on chromosome arm 1BS.

Overall, some new insights have been gained from the experiments presented in this dissertation. Improvement in yields under water-limited environments will naturally

follow as we better understand the genetics of root character traits and the relationships that they share with whole plant strategies. Hopefully these ideas proposed herein and the questions that were left unanswered will find interest and be worked to the point of resolve.

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