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Effect of Allelic Variation in Rht Loci on Plant Height and Grain Yield of Soft Red Winter Wheat (Triticum aestivum L.)

Habib Hayat
University of Arkansas, Fayetteville

Effect of Allelic Variation in *Rht* Loci on Plant Height and Grain Yield of Soft Red Winter Wheat (*Triticum aestivum* L.)

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Habibullah Hayat
Kabul University
Bachelor of Science in Agronomy, 2012

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University of Arkansas

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R. Esten Mason, Ph.D.
Thesis Director

Ainong Shi, Ph.D.
Committee Member

Andy Pereira, Ph.D.
Committee Member

David Miller, Ph.D.
Committee Member

Abstract

Plant height in wheat (*Triticum aestivum* L.) is controlled in large part by two major *Rht* genes, *Rht-B1* and *Rht-D1*, which pleiotropically impact lodging and grain yield. Prior to the Green Revolution, wheat varieties contained only 'wild-type' *Rht* alleles (*Rht-B1a* and *Rht-D1a*) and were tall and prone to lodging. Introgression of a semi-dominant mutation at either of these two loci (*Rht-B1b* or *Rht-D1b*) results in a semi-dwarf phenotype and reduced plant height. When combined (*Rht-B1b* and *Rht-D1b*) an extremely short double-dwarf phenotype is observed. The objective of this study was to determine the impact of allelic variation in *Rht-B1* and *Rht-D1* on plant height, grain yield, and yield components in soft red winter wheat (SRWW). A doubled haploid population (n = 98) derived from the lines 'Neuse' (*Rht-D1* dwarfing) and 'Bess' (*Rht-B1* dwarfing) segregating at the *Rht* loci, was evaluated in five total site-years in Arkansas. Analysis of variance across locations showed that allelic variation at the *Rht* loci significantly affected grain yield, plant height, and yield components ($p \leq 0.05$) with no *Rht* x location interaction. Overall, wild-type lines were taller (87.7 cm) and lower yielding (3.38 t ha^{-1}) compared to semi-dwarf lines. *Rht-D1* semi-dwarfs had significantly higher grain yield (3.93 t ha^{-1}) and were shorter (81.4 cm), compared to *Rht-B1* lines (3.72 t ha^{-1} and 83.3 cm). Higher grain yield in *Rht-D1* semi-dwarf lines was due in part to significantly higher 1000 kernel weight and kernel weight $spike^{-1}$, which resulted in higher kernel weight per spike. In addition, seven potential QTL associated with most of the traits measured were identified using a bi-parental approach. In conclusion, future breeding work should focus on the development of *Rht-D1* semi-dwarf lines adapted to Arkansas environment.

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Dedication

"Anyone who has never made a mistake has never tried anything new." - Albert Einstein

To the amazing people in my life who encourage and wish me to succeed further in my career especially to the people who tell me to go water my plants and flowers. Your presence and persuasion is what drives me to overcome any obstacle on my way. I will be forever grateful.

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Chapter I

Introduction and Literature Review

LITERATURE REVIEW

Wheat Production

Wheat (*Triticum aestivum* L.) is a staple crop across the globe, including in the United States (Acquaah, 2009), with 730.5 million tons produced in 2015 (Pocketbook 2015). According to the Crop Production Summary published by the United States Department of Agriculture (USDA), approximately 20.31 million hectares of agricultural land in the U.S. was used for wheat production in 2016, with a total production of 62.6 million tons and an average yield of 2.9 tons ha^{-1}. This included an area of 78 thousand hectares in Arkansas with 168.7 thousand tons of wheat produced at an average of 3.6 tons ha^{-1}.

Classes and Uses

There are six distinct classes of wheat based on kernel color (white vs red) hardness (hard vs soft) and planting season (winter vs spring) (http://www.uswheat.org/). Winter wheat is generally red and classified as either hard (high protein) in the case of hard red winter wheat (HRWW) or soft (low protein) in the case of soft red winter wheat (SRWW) (Knott 2007). Other classes include hard red spring wheat (HRSW), soft white wheat (SWW), hard white wheat (HWW), and durum wheat (McGaughey et al. 1990).

Winter wheat is planted in the fall and is followed by a dormancy period in the winter. Following dormancy, growth resumes in the spring with winter wheat harvested in the late spring or early summer. HRWW is used for bread flour and is predominantly grown in the Great Plains region. SRWW is used for pastries, cake and cookies, or blended in flour and is grown in the eastern US (Clark 2008).

Spring wheat, as the name suggests, is planted in early spring and harvested in late summer. As such it is less tolerant to low temperatures compared to winter wheat. HRSW is grown predominantly in the north central states and used for "designer" wheat products such as hearth breads, rolls, croissants, bagels and pizza crust and is the standard wheat for bread making. Hard and soft white wheat are spring wheat as well and are grown in western and Midwestern states. White wheat is used for Asian noodles, white bread, tortillas, and flat bread (Bridgwater and Sherwood 1959; Lukow 2006; Morris and Rose 1996; Rasiah et al. 2005; Tsilo et al. 2011).

Durum is the hardest of all wheat classes, with a strong amber color and high protein and gluten content. Durum is the ideal class of wheat for macaroni and premium pasta products. This class of wheat is widely grown in North and South Dakota, and Minnesota (Bennici 1986; Cleary and Brennan 2006; Cunin et al. 1995; Feillet and Dexter 1996).

Genetics of Wheat

Wheat (*Triticum aestivum*) is an allopolyploid species having the largest genome among the Poaceae family (William et al. 2007). The hexaploid bread wheat (*Triticum aestivum*) genome is comprised three subgenomes (ABD) that resulted from two hybridization events (Marcussen et al. 2014). The first hybridization is believed to have occurred ~ 10,000 years ago and brought together two diploid genomes, a relative of *Triticum Urartu* (2n=2x=14) with AA (A subgenome lineage), and another species *Aegilops speltoides* (2n=2x=14) with SS (B subgenome lineage). It resulted in the allotetraploid *Triticum turgidum* (2n=4x=28, AABB), a precursor of wild emmer and durum wheat. The second hybridization event occurred ~ 8000 years ago between *Triticum turgidum* and a diploid grass species *Aegilops taushii* (2n=2x=14) with DD (D subgenome

lineage) and gave rise to the allohexaploid *Triticum aestivum* (2n=2x=42) with AABBDD genome. Fertility of allopolyploid wheat was conferred by chromosome doubling (Kamran et al. 2014).

The large size of the wheat genome has hindered the development of a fully sequenced and physically ordered genome (Kamran et al. 2014). Despite this, development of a genome sequence is underway in addition to the development of other tools including: a whole-genome shotgun sequence, in which the entire genome is sheared into small sequence-able fragments and then reassembled (Brenchley et al. 2012); sequencing of the D genome ancestor *Aegilops taushii* (Jia et al. 2013), and; A genome ancestor *Triticum Urartu* (Ling et al. 2013).

Green Revolution

There have been many revolutions in agriculture which have impacted civilization, including learning agriculture ~11,500 BC, agricultural practices ~9,500 BC, fertilization ~3500 and rotation cropping ~6,000 BC (Borlaug 1976). The most recent began in the 1960s, spanning through the early 1970s and was known as the Green Revolution. The Green Revolution played a tremendous role in increasing grain productivity and putting an end to potential famine in Mexico, Pakistan, India, and the Philippines (Table 1).

During the mid-1960s scientists at the International Maize and Wheat Improvement Center (CIMMYT) worked to develop modern varieties of wheat for distribution to farmers in Latin America and South Asia.

The name Green Revolution refers to the success of these modern varieties. (Evenson and Gollin 2003). Norman Borlaug, the leading scientist behind the breeding program, wrote the following in his memoir in 1971:

“Civilization as it is known today could not have evolved, nor can it survive, without an adequate food supply. Yet food is something which is taken for granted by most world leaders despite the fact that more than half of the population of the' world is hungry. Man seems to insist on ignoring the lessons available from history.” (Borlaug 1971) He received the Nobel Prize for Peace for his efforts in 1970.

The key foundation to the Green Revolution was improving wheat genetic resources (Acquaah 2009). This revolution began through the introduction of a stem-shortening gene into wheat which led to the development of semi-dwarf, higher yielding crop varieties (Hedden 2003; Trethowan et al. 2007). Semi-dwarf varieties provided and advantage to farmers, as tall wild-type cultivars were not able to withstand the weight and pressure of the developing grain, causing stems to break and lodge. The origin of this height reducing (*Rht*) gene traces to japan in 1935, where a scientist named ‘Gonjiro Inazuka’ crossed a Japanese semi-dwarf landrace with two American cultivars, resulting in the cultivar ‘Norin 10’ (Lumpkin 2015). Norin 10 brought the typical height of 150 cm down to 60-110cm. It was brought to Washington State where Vogel developed the Norin 10 derived ‘Brevor’ cultivar that eventually ended up in the hands of Norman Borlaug. During early to mid-1950s, Borlaug crossed sources of the semi-dwarf phenotype with local Mexican varieties, resulting in the short and hard-stemmed varieties ‘Sonora 64’ and ‘Lerma Rojo 64’. Both varieties were high tillering, high yielding, and less prone to lodging (Lumpkin 2015). Semi-dwarfing alleles are now present in more than 70 % of globally cultivated wheat varieties. It would later be discovered Norin 10 contained two dwarfing genes which are partial-dominant alleles of homoeologous genes on the 4B and 4D chromosomes. The alleles *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) at these loci both have a similar effect on reducing height and are additive when combined. (Hedden 2003)

The Rht-B1 and *Rht-D1* loci

The two most prominent dwarfing genes in wheat are *Rht-B1* and *Rht-D1* located on chromosomes 4B and 4D, respectively (Börner et al. 1997; Gale and Youssefian 1985). The stem shortening alleles *Rht-B1b* and *Rht-D1b*, were introduced into wheat varieties developed during the Green Revolution to alleviate lodging and improve nitrogen uptake for higher grain yield (GY) (Pearce et al. 2011). They are now present in almost all wheat varieties which contains Norin10-Brevor in their pedigrees (Evans 1998). Peng et al. (1999) reported these genes to encode DELLA proteins, which are transcriptional regulators that repress gibberellic acid (GA) signaling. GA is responsible for that regulating developmental growth in plants. The *Rht-B1b* and *Rht-D1b* allele sequences are each polymorphic in a single nucleotide in comparison to the wild type alleles (Figure 1), *Rht-B1a* and *Rht-D1a*, which introduces a premature stop codon in the N-terminal coding sequence and results in dwarfism caused by suppression of GA signaling.

The reduced height alleles modify the morphology and physiology of the plant while compensating for many physiological processes such as decreasing leaf area but increasing photosynthesis per unit area, increasing leaf permeability to vapor but changing the water condition to accommodate for efficient use, and greater accumulation of carbohydrates during shoot elongation (Gent and Kiyomoto 1997). Guedira et al. (2010) reported that all U.S. wheat varieties developed before 1964 had the wild type allele at both loci. However, the frequency of the *Rht-B1b* and *Rht-D1b* alleles increased sharply after their introduction, with 90% of modern cultivars having a semi-dwarf growth habit. In Guedira et al. (2010) study, the *Rht-D1b* allele was present in 45% of SRWW varieties, compared to 28% for *Rht-B1b*. In contrast, *Rht-B1b* was present in 77% of hard winter wheat cultivars.

Other Known *Rht* Genes

In addition to *Rht-B1b* and *Rht-D1b,* other GA insensitive alleles include *Rht-B1c* (*Rht3*) and *Rht-D1c* (*Rht10*) (Gale and Youssefian 1985). The source of *Rht-B1c* is quite uncertain but Zeven (1969) traced it to the variety 'Tom Thumb' whose parents were 'Tom Pouce Blanc' and 'Tom Pouce Barbu Rouge' and date back to the British variety 'Hybrid Carter G.'. Using bulk segregant analysis, Navarro et al. (2014) mapped this gene to chromosome 4B and Wen et al. (2013) concluded that *Rht-B1c* has partially dominant and co-dominant effect on plant height, showing increased dwarfing in wheat. *Rht-D1c* is a more severe dwarfing allele of GA insensitive *Rht-D1b* allele (Casebow et al. 2016). Izumi et al. (1981) analyzed this allele in a cross between 'Ai-bian' and an *Rht-D1b* genotype and localized this gene to the short arm of chromosome 4D. They reported that lines possessing the *Rht-D1c* allele were 2-5cm shorter than lines with *Rht-B1c*.

Another major group of genes known to be responsible for height reduction are GA sensitive genes (Gale and Youssefian 1985). The 'Akakomugi' genes, including *Rht8* and *Rht9,* were first discovered in Italian wheat varieties (Borojevic and Borojevic 2005; Law 1983). Korzun et al. (1998) located the *Rht8* and *Rht9* genes to the short arm of chromosome 2B, and short arm of chromosome 7B, respectively. While initially *Rht8* was shown to have no effect on early growth of wheat in comparison to *Rht-B1 and Rht-D1* (Ellis et al. 2004), a later study by Amram et al. (2015) found *Rht8* to have a significant positive impact on emergence and grain yield at variable planting depths. Their results indicated that varieties containing alleles for GA insensitivity (including *Rht8*) had a significantly shorter coleoptile compared to GA sensitive varieties, each at 8.6 cm and 12.4 cm, respectively. On the other hand, they found that the emergence time was the contrary at 11.87 days for GA insensitive vs. 9.16 days for GA sensitive

lines under controlled conditions. Their study also showed that a sowing depth of 10 cm, compared to a control of 2 cm, decreased GY by 66.7 and 33.9% in GA insensitive and sensitive lines, respectively. It was concluded that GA sensitivity (including *Rht8*) is preferred for deep sowing, with least amount of decrease in GY.

Additional *Rht* genes include *Rht4*, *Rht5*, and *Rht7*. *Rht4* was a mutation induced by gamma rays and reduced the height of variety 'Burt' up to 45%(Gale and Youssefian 1985; Hu 1980). *Rht5* was the result of ethyl methyl sulphonate treatment and reduced plant height by 50% but has little commercial value (Gale and Youssefian 1985; Woo and Konzak 1969). *Rht7* was also the result of ethyl methyl sulphonate treatment of the variety "Bersée" and is reported to be located on chromosome 2A. Due to complications, such as low GY, it was concluded that *Rh7* is of little or no use in breeding programs (Gale and Youssefian 1985; Worland et al. 1980).

QTL mapping

Quantitative trait loci (QTL) are regions of a genome responsible for variation in quantitative traits (Doerge 2002). QTL mapping is the experimental estimation of marker mean and variance associated with a locus. It depends on changes between trait means of different genotypes at a locus (Bernardo 2008). QTL mapping involves a segregating population, its genotypic data with molecular markers, phenotypic data for traits of interest, and statistical procedures to detect markers related to QTL (Bernardo 2002; Lynch and Walsh 1998; Udall 2003). For marker means, assuming M is a marker locus, *r* is recombination frequency, Q is QTL, the genotypic value for QQ is P + α, Qq is P + d, and qq is P – α. In a doubled haploid (DH; without heterozygotes) population derived from a cross of MMQQ and mmqq, the means of MM and mm genotypes in the F1 generation are:

$$MM = P + \alpha(1 - 2r)$$

$$mm = P - \alpha(1 - 2r)$$

They give the following difference between the means of marker genotypes:

$$(MM - mm) = 2\alpha(1 - 2r)$$

For Recombinant Inbred line (RIL) populations, recombination frequency is $R = 2r/(1 + 2r)$ and *R* replaces *r* in the calculations (Bernardo 2002; Cowen 1988; Doerge 2002).

There are multiple methods for conducting QTL mapping such as single marker analysis (SMA), interval mapping (IM), composite interval mapping (CIM), and multiple interval mapping (MIM). SMA uses a *t*-test, ANOVA or simple linear regression to assess a phenotype linking to a genotype, indicating marker trait association, hence, exhibiting potential QTL. Usually, H_0 = mean of trait being independent of genotype at a specific marker. It is rejected when the test statistic is bigger than a critical value, meaning a QTL is associated to a tested marker. SMA is typically used for detecting single markers such as disease resistance instead of investigating genomic regions. SMA cannot provide the location of QTL relative to marker because of *r* (e.g., 1-2*r*) cofounding with genotypic value (2α). In addition, it is limited in detecting two or more flanking markers as independent QTLs. A segment of a chromosome between two adjacent markers is a marker interval. Interval mapping solves for confounding effects by estimating both the location of a QTL and QTL effect between flanking markers. IM uses maximum likelihood to calculate the genetic distance and location of markers, in centiMorgans (cM), on a chromosome. It also calculates a logarithm of odds (LOD) score which is a likelihood-ratio divided by 2 ln 10. An LOD score of 3.0, which is equal to an odds of 1000:1, is common as a threshold for reporting the presence of a QTL. IM's limitation is

exhibition of ghost QTL between two flanking markers leading to false positives. CIM utilizes both IM and multiple regression. It locates the QTL between a pair of flanking markers using interval mapping and uses multiple regression to account for QTL effects elsewhere in the genome as co-factors in the regression analysis. CIM is more robust than IM for using other QTLs to control appearing of background variation or ghost QTL. MIM creates a multiple-QTL model by accounting for several markers at once. MIM uses a step by step procedure for detecting potential QTL: 1) Each QTL is fitted successively in the model; 2) Searches for epistasis in the effect of individual QTL; 3) Adjusts the effect of those individual QTL of any errors; 4) Adjusts the location estimation for each of the QTL linking to its closest flanking markers. The step is repeated until desired stability of the estimates. MIM differs from CIM in using the potential or putative QTL as co-factors for each other QTL instead of using background markers. Many current statistical software are able to perform these mapping approaches such as WinQTLCart, QGene and MAPMAKER/QTL (Bernardo 2002, 2008; Doerge 2002; Kao et al. 1999; Lincoln et al. 1993; Nelson 1997; Silva et al. 2012; Wang et al. 2002; Zeng 1994)

OBJECTIVES AND HYPOTHESES

The *main objective for this study* is to compare the genetic effect of allelic variation in the height reducing genes *Rht-B1* and *Rht-D1* on wheat plant height and GY in SRWW. The results will have a significant impact on criteria for selecting parents for future breeding. The specific objectives of the proposal are:

a) Objective 1: Determine the impact of allelic variation at the *Rht-B1* and *Rht-D1* loci on GY, yield components and plant height in a doubled haploid (DH) population segregating at these loci. The hypothesis is that semi-dwarf lines have higher GY compared to both

double dwarfs and wildtypes. In addition, we hypothesize that semi-dwarfs with *Rht-D1b* will yield higher compared with semi-dwarfs having *Rht-B1b* alleles.

b) Objective 2: Identify additional QTLs for plant height, heading date, and GY using a bi-parental mapping approach. We hypothesize that this approach will identify additional QTL conferring reduced plant height and higher GY.

Justification of the research project

Semi-dwarfing in wheat is classified as the result of either *Rht-B1* or *Rht-D1* dwarfing loci. The former has the *Rht-B1b*/*Rht-D1a* alleles and the latter has the *Rht-B1a*/*Rht-D1b* alleles. Varieties containing *Rht-B1* semi-dwarfing are grown predominantly in the northern United States. *Rht-D1* semi-dwarfs are grown mostly in the south and southeastern regions of the U.S. The geographical location of the state of Arkansas borders the northern and southeastern regions of the U.S. This project was designed to determine whether or not to integrate *Rht-D1* semi-dwarf lines into the available germplasm, through testing the two *Rht* semi-dwarf haplotypes for GY and PH performance. A secondary objective was to have coherent collaboration in research and development of wheat varieties with the rest of the southeastern conference.

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Zeven A (1969) Tom Pounce Blanc and Tom Pounce Barbu Rogue, two Triticum aestivum sources of very short straw. Wheat Inform Serv

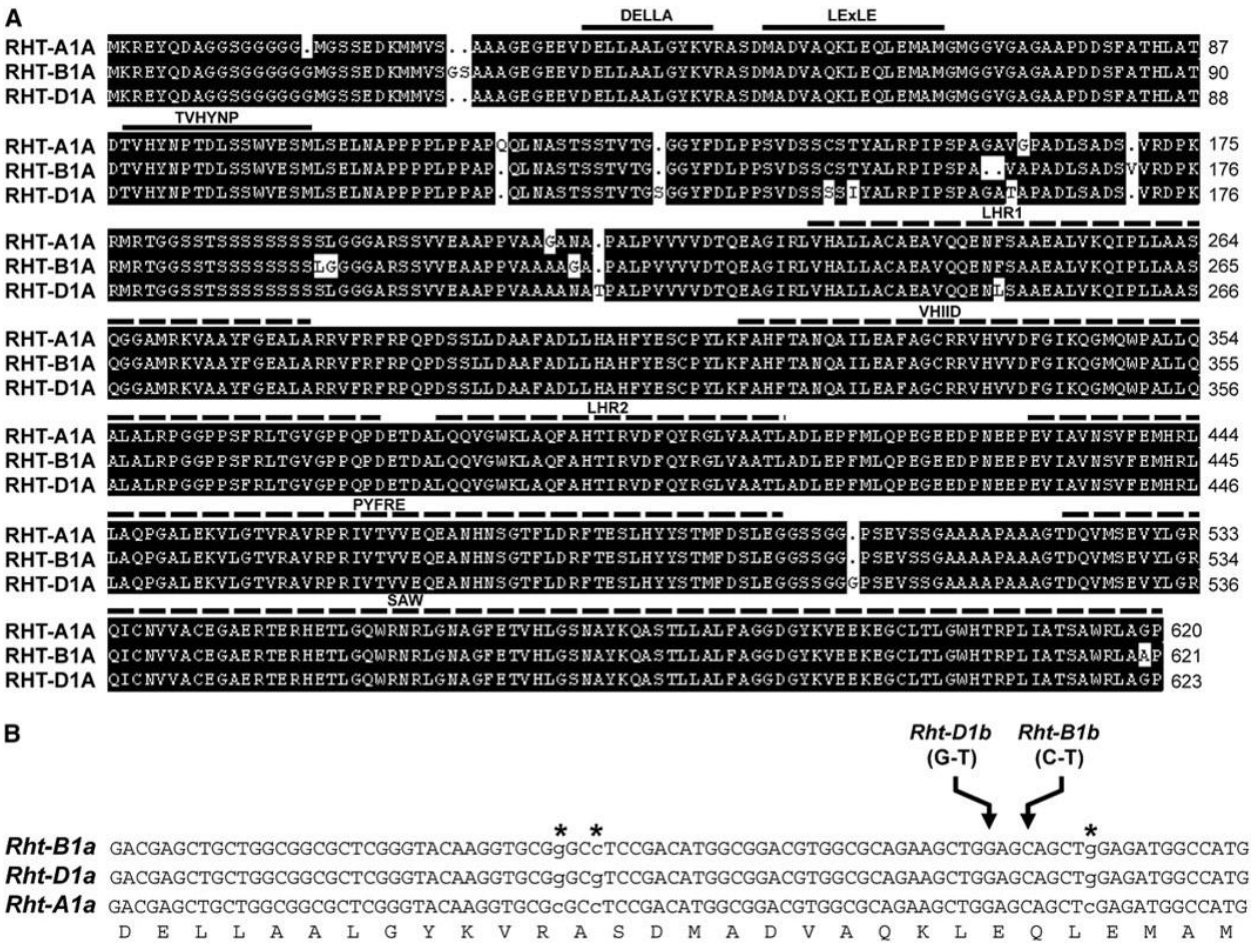
Table 1. World grain production 1960-1995

Year	Total grain (million tons)	Grain per capita (kg)
1960	847	279
1970	1096	296
1980	1447	325
1985	1664	343
1986	1683	341
1987	1612	321
1988	1564	306
1989	1685	324
1990	1780	336
1991	1696	315
1992	1776	316
1993	1703	307
1994	1745	309
1995	1680	293

Source: (Khush 1999)

Table 2. Traits' measurements and calculations.

Trait	Measurement
Plant height	From two measurement plot^{-1} at maturity in cm
Days to heading	50% of head visible in a plot in Julian days
Grain Yield	Whole plot combine t ha^{-1}
Test Weight	Whole plot combine in Kg hl^{-1}
Kernel weight spike^{-1}	50 spike grain weight/50 in g
Thousand kernel weight	The weight of thousand kernels in g
Kernel number spike^{-1}	(Kernel weight spike-1)/(1000 Kernel weight/1000)
Spike density	Grain yield (g m-2)/Kernel weight spike^{-1}



(Rajaram 1995)

Figure 1: Sequence of the wheat genome corresponding to the *Rht* genes.

Chapter II

Impact of Allelic variation in height reducing (*Rht*) loci on Grain Yield and Plant Height

Abstract

The objective of this study was to determine the impact of allelic variation in the Green Revolution reduced height loci, *Rht-B1* and *Rht-D1*, on grain yield (GY), plant height (PH) and yield components thousand kernel weight (TKW), kernel weight spike^{-1} (KWS), kernel number spike^{-1} (KNS) using a doubled haploid (DH) population segregating at these loci. Field trials were conducted in five total site-years over three growing seasons in Arkansas. The *Rht* loci significantly affected PH with double dwarfs (*Rht-B1b*/*Rht-D1b*) having the shortest stature compared to both *Rht-B1* and *Rht-D1* semi-dwarfs and wild-types (*Rht-B1a*/*Rht-D1a*). *Rht* loci had a significant effect on GY. *Rht-D1* semi-dwarfs had the highest yield of 3.93 t ha^{-1} compared to double-dwarfs (3.76 t ha^{-1}), *Rht-B1* semi-dwarfs (3.72 t ha^{-1}) and wild-types (3.38 t ha^{-1}). Trends were observed for both PH and GY across locations, with *Rht-D1* semi-dwarfs being shorter in stature compared to *Rht-B1* semi-dwarfs, with the exception of Newport 2015-16 (Npt16). *Rht-D1* semi-dwarfs were also higher yielding in all site-years compared to *Rht-B1* semi-dwarfs with a significant difference observed in Npt16 and Fayetteville 2016-17 (Fay17). Increases in total GY were due in part to significant increases in TKW and KWS. There was no significant interaction observed between the *Rht* loci and site-year for any of the traits measured. GY had a negative correlation with PH ($r = -0.30$, $P \leq 0.01$). PH was found to have a negative correlation with all measured traits with the exception of test weight ($r = 0.19$ and $P \leq 0.05$) and TKW ($r = 0.24$ and $P \leq 0.05$). Weekly measurement of PH was performed for Fayetteville 2016-2017 (Fay17) site-year. It showed significant differences between wild-types and both semi-dwarfs in at least four of the seven measurements. No significant difference was observed for double-dwarfs and other haplotypes possibly due to low frequency of double-dwarfs in the population. QTL mapping of the DH population found additional major or stable loci concerning

GY, TW, DTH, PH, KWS, and KNS. No stable QTL was found for KWS and SD (spike density).

Introduction

Wheat is a staple food crop, providing more than one fifth of the daily calorie needs around the world and grown on more than 17% of arable land (FAOSTAT 2017). To meet the increasing demand, grain yield (GY) improvement continues to be the major target for wheat breeding programs (Parry et al. 2010; Reynolds et al. 2009). GY is a complex trait controlled by genetic and environmental factors (Ashfaq et al. 2003) and therefore a holistic approach that incorporates agronomics (Wang et al. 2012), physiology (Foulkes et al. 2010) and genetics (Foulkes et al. 2010) is necessary for yield improvement.

The Green Revolution remains the most rapid increase in GY seen for agricultural crops. Norman Borlaug joined CIMMYT in 1944 with goal to make Mexico self-sufficient in wheat production (Rajaram 1995). After sixteen years of work, his initial goal of rust resistant wheat was met but he encountered lodging problems in his varieties (Lumpkin 2015). He found the solution in the variety 'Norin 10-Brevor 14', developed at Washington State University by Orville Vogel. Vogel had brought the predecessor ('Norin 10') from Japan. Norin 10 had two dwarfing alleles, including *Rht-B1* and *Rht-D1* on chromosomes 4B and 4D, respectively (Hedden 2003). Borlaug hybridized Norin10-Brevor 14 with his rust resistant but tall varieties. The results were 'Sonora 64' and 'Lerma Rojo 64'. These varieties were both rust resistant and provided lodging resistance despite the increased input of nitrogen fertilizers (Lumpkin 2015).

The *Rht-B1* locus on chromosome 4B and *Rht-D1* locus on chromosome 4D each have the possibility of a wild-type (a) or dwarfing (*b*) allele. In total, there are four possible haplotype combinations, including wild-type, *Rht-B1* semi-dwarfs, *Rht-D1* semi-dwarfs and double dwarfs. The dwarfing alleles *Rht-B1b* and *Rht-D1b* encode DELLA proteins which repress gibberellic acid (GA) signaling, resulting in GA insensitivity and reduced height in plants (Peng et al. 1999).

Both the *Rht-B1b* and *Rht-D1b* alleles cause similar reduction in plant height (PH). Gale and Youssefian (1985) and Allan (1997) found the *Rht-B1b* and *Rht-D1b* alleles to cause a 15 and 24% reduction in plant height, respectively. Comparing *Rht-B1* and *Rht-D1* semi-dwarfs, Flintham et al. (1997) found reductions in PH of 14 and 17%, respectively. In another study, *Rht-B1* semi-dwarfs were found to have a reduction in PH of 36% compared to wild-type (Trethowan et al. 2002).

Several studies have shown differential GY performance based on the segregation of *Rht-B1* and *Rht-D1* loci. Gale and Youssefian (1985) observed an association between *Rht-B1* and *Rht-D1* semi-dwarfing alleles and increased floret fertility with floret fertility also adversely affected by other *Rht* alleles such *Rht8*. It was later discovered that semi-dwarfing alleles increased partitioning of assimilates to generative parts of the plant instead of vegetative parts (Flintham et al. 1997). Knott (1986) and McNeal et al. (1972) both found no significant difference between both *Rht-B1* and *Rht-D1* semi-dwarfs compared to wild-types, with semi-dwarf lines having lower GY in low fertility fields. In contrast, others have observed GY increases of 24% (Flintham et al. 1997) and 16% (Allan 1986; Singh et al. 2001) for the semi-dwarfs, respectively. Chapman et al. (2007) reported a 21% increase in GY of *Rht-B1* semi-dwarfs; and Blake et al. (2009) and Chapman et al. (2007) showed an increase of 30 and 18% in GY due to *Rht-D1* semi-dwarfing compared to wild-types. Kuchel et al. (2007) and Robbins (2009) showed that lines with *Rht-B1* and *Rht-D1* semi-dwarfing were, on average, 150 kg ha^{-1} and 124 to 202 kg ha^{-1} higher yielding compared to wild-types, respectively. Butler et al. (2005) studied the two semi-dwarfing haplotypes in three different moisture levels, full irrigation, partial irrigation and rain-fed conditions and concluded that *Rht-B1* semi-dwarfs outperformed *Rht-D1*

semi-dwarfs under full irrigation but the two performed similarly in partial irrigation and rain-fed conditions.

Quantitative trait loci (QTL) mapping and analysis are tools use to assist plant breeders in selecting for potentially higher quality varieties (Addison et al. 2016; Vinod 2010). QTLs for PH were first reported by Allan et al. (1959) in Norin-10 and Tom Thumb. They discovered that these varieties were shorter than tall varieties and were not responsive to GA application. It was later confirmed that Norin-10 had *Rht-B1* (formerly *Rht1*) and *Rht-D1* (formerly *Rht2*) loci (Gale and Youssefian 1985). Molecular mapping of these loci were reported by several studies (Börner et al. 1997; Ellis et al. 2005; Pearce et al. 2011; Wen et al. 2013) locating them on chromosome 4B and 4D on the wheat genome. . Ellis et al. (2002) developed Polymerase Chain Reaction (PCR) based markers for *Rht-B1b* and *Rht-D1b* alleles. They successfully reported mapping the markers to loci homoeologous with *Rht-B1* and *Rht-D1* genes on chromosomes 4B and 4D, respectively.

Apart from their effect on PH, *Rht-B1* and *Rht-D1* loci were studied extensively for their pleiotropic effect on agronomic traits (Allan 1986) such as grain yield and yield components (Flintham et al. 1997; Gent and Kiyomoto 1997; Kertesz et al. 1991), grain quality (Casebow et al. 2016), early vigor (Botwright et al. 2005), days to heading (DTH) (Wilhelm et al. 2013), soil moisture levels and irrigation (Butler et al. 2005), vegetative growth (Youssefian et al. 1992), and herbicide resistance (Gale and Youssefian 1983).

Most of the above mentioned studies addressed the impact of allelic variation in *Rht* loci in spring wheat varieties and populations. Very few studies have been performed using winter wheat populations or germplasms adapted to the southeastern U.S. Therefore, the objective of this study was to determine the impact of allelic variation in the Green Revolution reduced

height (*Rht*) loci, *Rht-B1* and *Rht-D1*, on grain yield (GY), plant height (PH) and yield components using a doubled haploid (DH) population segregating at these loci. For this study, we hypothesize that semi-dwarf lines yield higher compared to wildtypes and double dwarfs. Further, we hypothesized that *Rht-D1* semi-dwarfs will have increased grain yield in contrast to *Rht-B1* semi-dwarfs. Lastly, we hoped to detect any additional QTL affecting GY, GY components, DTH, and PH through a bi-parental mapping approach.

Materials and methods

Doubled haploid population

The population under study consisted of 98 soft red winter wheat (SRWW) doubled haploid (DH) lines derived from two SRWW varieties, 'Bess' and 'Neuse'. Bess was developed by the University of Missouri and was released in 2005 (McKendry et al. 2007). The variety is resistant to *Fusarium* head blight (FHB) and moderately resistance to stripe rust but is susceptible to stem rust and leaf rust. Its accession number is PI-642794 and its pedigree is: (MO-11769/MADISON). Its extensive pedigree is available at (http://wheatpedigree.net/sort/renderPedigree/83643). Bess is a semi-dwarf and contains the dwarfing allele *Rht-B1b* at the *Rht-B1* locus and the wild-type allele *Rht-D1a* at the *Rht-D1* locus. Due to the presence of the *Rht-B1b* allele, Bess has gibberellic acid (GA) insensitivity and semi-dwarf morphology. It also contains the photoperiod sensitive allele *Ppd-B1b* at the *Ppd-B1* locus, located on chromosome 2B (Petersen et al. 2016).

Neuse was developed by North Carolina State University and was released in 2003 (Murphy et al. 2004). It has moderate susceptibility to FHB, susceptibility to stripe rust, moderate resistance to leaf rust and resistance to powdery mildew. Its accession number is PI-633037 and

its pedigree is: (COKER-86-29//STELLA/CHD-756-80/3/COKER-9907). Its extensive pedigree is available at (http://wheatpedigree.net/sort/renderPedigree/82784). Neuse is semi-dwarf and has the wild-type allele (*Rht-B1a*) at the *Rht-B1* locus, the dwarfing allele (*Rht-D1b*) at the *Rht-D1* locus and a null allele for *Ppd-B1* locus (Petersen et al. 2016).

Both parents have the Norin10-Brevor 14 cultivar in their pedigree, which is historically the main source of *Rht-B1b* and *Rht-D1b* dwarfing alleles. Both are well adapted to the southeastern United States and have a *Ppd-A1a* (insensitivity) allele at the *Ppd-A1* locus and the *Ppd-D1b* (sensitivity) at the *Ppd-D1* locus. The DH population has previously been studied for Fusarium head blight (FHB) resistance (Petersen et al. 2016). The DH population segregates at the height reducing loci *Rht-B1* and *Rht-D1*, on chromosomes 4B and 4D, respectively. At both loci there are two possible alleles, a dwarfing allele (indicated by '*a*') and a wild-type or tall allele (indicated by '*b'*). Collectively there are four allele combinations or haplotypes. In the population, there are 35 semi-dwarf lines that possess the *Rht-B1b* (dwarfing) and *Rht-D1a* (wild-type) haplotype, 50 semi-dwarf lines which possess the *Rht-B1a* (wild-type) and *Rht-D1b* (dwarfing) haplotype, 8 lines which are wild-type or tall at both loci (*Rht-B1a* and *Rht-D1a*) and two lines which possess dwarfing alleles at both loci (*Rht-B1b* and *Rht-D1b)* or double-dwarf. For ease of reference, the first haplotype will be referred to as *Rht-B1* semi-dwarf; the second haplotype will be referred to as *Rht-D1* semi-dwarf; the third haplotype will be referred to as wild-type; and the fourth haplotype will be referred to as the double-dwarf for the remainder of this thesis.

Experimental locations and design

The DH population and parents were evaluated over three growing seasons in Fayetteville 2014-15 (Fay15), Fayetteville 2015-16 (Fay16) and Fayetteville 2016-17 (Fay17); and Newport

2015-16 (Npt16) and Newport 2016-17 (Npt17) in Arkansas for five total site-years. Fayetteville sites consisted of Captina soil which is classified as fine-silty, siliceous, active, mesic Typic Fragiudults. Newport sites consisted of Calhoun-Foley soils. Calhoun soil is classified as fine-silty, mixed, active, thermic Typic Glossaqualfs. Foley soil is classified as fine-silty, mixed, active, thermic Albic Glossic Natraqualfs (Nrcs 2009).

The population was planted in a randomized complete block design with the two parental lines used as repeated checks. Each site-year consisted of two replications with the exception of Fayetteville (2014-2015), which had only one replication. All locations were drill-seeded at ~ 118 kg seed ha^{-1} with plot dimensions of 1.52 meters wide and 4.26 meters long. The sowing date for the 2014-2015 season was October 25, 2014 for Fayetteville, AR. Fayetteville hasFor 2015-2016 season, sowing occurred on October 21, 2015 in Fayetteville and November 10, 2015 in Newport, AR. For the 2016-2017 season, sowing occurred on October 18, 2016 in both Fayetteville, AR and Newport, AR. Plots were harvested during May and June depending on physiological maturity at each location. Nitrogen was applied to both locations in a split application (100.87 kg ha^{-1} and 67.45 kg ha^{-1}) beginning at Feekes Growth Stage 5. For pest control and management, herbicides including Harmony® (DuPont™) for controlling winter annual weeds, Axial® (Syngenta Group Company) for controlling ryegrass (*Lolium persicum*), Osprey® (Bayer) for controlling fully grown Italian ryegrass (*Lolium multiflorum*) during 2015-2015 season, Grizzly® (Winfield Solutions, LLC) for pest control, and a fungicide Tilt® (Syngenta Group Company) for foliar disease control, were applied at the recommended rates and proper growth stages.

Data Collection

Plant height (PH) was measured at physiological maturity twice per plot from the soil surface to the top of each plot, excluding awns and averaged for one value per plot. In Fayetteville 2016-2017, PH was also measured weekly beginning at Feekes 4 until full height. Days to heading (DTH) was measured in Julian days count when approximately 50% of heads in a plot were completely visible. GY was determined at all locations by whole plot harvesting and adjusting to 13% moisture in tons ha^{-1}. For 2016-2017, yield components including thousand kernel weight (TKW), kernel weight $spike^{-1}$ (KWS), and kernel number $spike^{-1}$ (KNS), were measured by randomly harvesting 50 spike-bearing culms from each plot according to Reynolds (2001). Test weight (TW) was measured on a volume basis of kg hl^{-1}.

Statistical analyses

The phenotypic data were analyzed using PROC MIXED in SAS 9.3 (SAS Institute, Inc., 2011, Cary, NC) with all factors (*Rht*, location, replication, *Rht* * location, replication(location)) as fixed effects for analysis of variance (ANOVA) and testing for significance. TYPE 3 sums of squares were used for estimating narrow sense heritability of traits from ANOVA with all factors treated as random effects using the following formula:

$$h^2 = \frac{\sigma^2_{genotype}}{\left(\sigma^2_{genotype} + \sigma^2_{\frac{genotype \times location}{l}} + \sigma^2_{\frac{residual}{l \cdot r}}\right)}$$

In which $\sigma^2_{genotype}$ is the genotypic variance, $\sigma^2_{genotype \times location}$ is the interaction of genotype and environment (G × E) variance, and $\sigma^2_{residual}$ is the error variance. The *l* and *r* are the number of locations and replication, respectively.

Genotypic data and QTL analysis

A genetic map of the DH population with 6674 Illumina (San Diego, CA) 9K *iSelect* SNP assay markers (Cavanagh et al. 2013) previously developed by Petersen et al. (2016) was used for QTL analysis. The DH population was also genotyped for diagnostic KASP markers for *Rht-B1* and *Rht-D1*, as previously reported by Petersen et al. (2016). A single marker analysis was conducted to determine the effect of allelic variation in *Rht-B1* and *Rht-D1* on grain yield, yield components and agronomic traits in both the DH population. For the identification of additional quantitative trait loci (QTL) in the DH population, composite interval mapping in WinQTLCart v. 2.5 was used (Silva et al. 2012). The LOD threshold was set to 2.5 and only QTLs detected in at least two environments and the combined analysis were declared stable (Addison et al. 2016). The illustration of linkage groups was performed in Mapchart v.2.2 (Voorrips 2002).

Results

Phenotypic performance

Bess had higher grain yield than Neuse in all five site-years (Table 1). The DH lines, on average, yielded higher than Neuse in Fay16, Npt16, and Npt17 but had lower grain yield compared to Bess in all site-years. For all the site-years, Bess had lower TW compared to Neuse. The DH lines had lower mean TW (72.3 kg hl^{-1}) compared to Neuse (78.1 kg hl^{-1}) and Bess (77.5 kg hl^{-1}) in all the site-years with the exception of Fay16. For PH, Bess was on average, 3.8 cm taller than the Neuse across all site-years. On the other hand, the DH lines were, on average, 1.5 cm shorter than Neuse and 5.3 cm shorter than Bess. Data for DTH were collected in Fay15 and Fay17 with similar results observed for Neuse (98.4 days) and Bess (98.5 days) but numerically higher (100.0 days) results for DH lines.

Analysis of variance and correlation for site-years and traits

Both *Rht* and location had a significant ($P \leq 0.001$) effect on GY, PH, KWS, TKW and KNS (Table 2) while no significant interaction between *Rht* and site-year was observed. Narrow sense heritability ranged from $h^2 = 0.60$ for KWS to $h^2 = 0.94$ for PH. The heritability of GY was $h^2 = 0.82$ across the five site-years.

GY was positively correlated with all measured traits with the exception of PH where a negative correlation was observed ($r = -0.30$ and $P \leq 0.01$). TW showed a positive correlation with all traits with the exception of DTH ($r = -0.22$ and $P \leq 0.05$). PH had a negative correlation with all measured traits with the exception of TW ($r = 0.19$ and $P \leq 0.05$) and TKW ($r = 0.24$ and $P \leq 0.05$). GY and PH had a highly significant negative correlation ($r = -0.30$ and $P \leq 0.01$) which indicated higher yield was associated with shorter stature.

Effect of *Rht* haplotypes on GY and yield components

Variation in *Rht* loci significantly impacted GY, with *Rht-D1* semi-dwarf lines having significantly higher mean GY (3.93 t ha^{-1}) compared to double-dwarfs (3.76 t ha^{-1}), *Rht-B1* semi dwarfs (3.72 t ha^{-1}) and wild-type lines (3.38 t ha^{-1}) (Figure 1). The GY of wild-type lines was significantly lower than all other haplotypes. Comparing the performance of *Rht-B1* and *Rht-D1* semi-dwarfs across locations showed a trend of *Rht-D1* semi-dwarf lines having higher GY compared to *Rht-B1* in all five site-years, with significantly higher GY observed in Npt16 and Fay17 (Figure 2). In addition to GY, *Rht-D1* semi-dwarf lines had significantly higher TKW (31.62 g) compared to *Rht-B1* semi-dwarf lines (30.60 g) (Figure 3a) and significantly higher KWS (0.93 g), compared to *Rht-B1* semi-dwarf lines (0.90 g) (Figure 3b).

Effect of *Rht* haplotypes on plant height (PH)

Similar to GY, significant variation in PH was also observed among the haplotypes of *Rht* loci (Figure 5). Across all five site-years, *Rht-D1* semi dwarfs were significantly shorter (81.4 cm) compared to *Rht-B1* semi-dwarf (83.3 cm) and this trend was consistent across site-years with the exception Npt16, with a significant difference observed in Fay15 and Npt17 (Figure 6). The tallest semi-dwarfs were observed in Npt17 and Fay17 site years. In Fay17, *Rht-B1* semi-dwarfs averaged 88.6 cm compared to 86.6 cm for *Rht-D1* semi-dwarf lines. Npt17 showed similar results in PH with *Rht-B1* semi-dwarfs having a mean of 88.7 cm compared to 85.5 cm for *Rht-D1* semi-dwarfs. The lowest PH was observed in Npt16 where *Rht-B1* semi-dwarfs were on average 70.9 cm compared to *Rht-D1* semi-dwarfs being 71.6 cm on average.

Weekly PH measurements were taken for Fay17 beginning at Feekes 4 stage (Figure 7). Significant differences were observed between wild-types and *Rht-D1* semi-dwarfs for all seven measurements and in four of the seven for *Rht-B1* semi-dwarfs compared to wild-types. A significant different was observed between *Rht-B1* semi-dwarfs and *Rht-D1* semi-dwarfs in four of the seven measurements. No significant difference was observed between double-dwarfs and other haplotypes. At Feekes growth stage 4, *Rht-D1* semi-dwarfs had the shortest PH (35.6 cm) and wild-types the tallest (40.1 cm). *Rht-D1* was numerically shorter compared to double-dwarfs in three of the seven measurements. The double-dwarfs remained the shortest for the remainder of the growing season with a final height of (78.0) cm which was followed by *Rht-D1* semi-dwarfs lines, *Rht-B1* semi-dwarfs, and wild-types at 82.8 cm, 87.6 cm, and 87.6 cm, respectively.

QTL for Grain yield (GY), test weight (TW) and days to heading (DTH)

For GY a cluster of three individual QTL (*QYld.ua-3Ba*, *QYld.ua-3Bb*, and *QYld.ua-3Bc*) were detected in the 4.0 to 30.0 cM region of the chromosome 3B.1 with highest LOD of 4.46 and lowest LOD of 2.98 which explained 10 to 12% of the phenotypic variation. Neuse provided the first allele with negative additive effect while Bess provided the favorable alleles with positive additive effects. Related genotypic markers were: *IWB6207*, *IWB35069*, and *IWB34153*.

Two stable QTL were discovered for TW. *QTw.ua-3A* was located on chromosome 3A at 41.3 cM with maximum LOD of 5.49 near the genotypic marker *IWB5723* and explained 7 to 23% of the phenotypic variation. Its favorable allele was provided by Bess. The second stable QTL, *QTw.ua-6A*, was detected at 11.3 cM on chromosome 6A with a maximum LOD of 5.46 and explained up to 21% of the phenotypic variation and unfavorable allele coming from Neuse. Three QTL for DTH were detected. *QDth.ua-3B* was identified at 114.1 cM on chromosome 3B.2 with an LOD of 4.42 explaining 19% of the phenotypic variation with favorable allele coming from Neuse. It was localized between two flanking markers: *IWB47459* and *IWB57820*. A second QTL, *QDth.ua-6B*, was detected at 8.3 cM on chromosome 6B with an LOD of 4.27 near genotypic marker *IWB8078*. *QDth.ua-6B* locus had additive effects from Bess, meaning Neuse provided the favorable allele at this QTL. A third QTL, *QDth.ua-7B*, for DTH was identified at 5 cM on chromosome 7B with an LOD of 3.23. *QDth.ua-7B* was observed with negative additive effects coming from Neuse. Alleles providing decreased DTH were counted as favorable for DTH in the DH population. It was located between two flanking markers: *IWB10879* and *IWB35941* (Figure 8; Table 12).

QTL for GY components and plant height (PH)

Due to missing data for three site-years (Fay15, Fay16, and Npt16), LSMEANS were used to detect stable QTL for GY components and were chosen for their co-localization with at least one other GY component. One major region of the genome with stable QTL for GY components was found at 94.2 cM on chromosome 4A. *QKws.ua-4A* and *QKns.ua-4A* were detected with an LOD of 4.69 explaining up to 13% of phenotypic variation with favorable allele coming from Bess. They were located near *IWB71809* genotypic markers.

In addition to the *Rht-B1* and *Rht-D1* loci, a major stable QTL for PH was detected on chromosome 3B.2. This region contained four individual QTL, *QPh.ua-3Ba-d*, which had LODs ranging from 4.16 to 5.92 with R^2 values of up to 0.19. *QPh.ua-3Ba-d* had positive additive effects from Bess pointing to favorable alleles coming from Neuse.

Discussion

Impact of *Rht* loci on grain yield (GY)

Reduced height genes have been shown to have a significant effect on GY, yield components, and PH (Butler et al. 2005; Robbins 2009). Kuchel et al. (2007) analyzed a DH population of Australian winter wheat segregating for *Rht-B1* and *Rht-B1* loci and showed that the semi-dwarf lines had 0.15 t ha^{-1} more GY than the wild-type. Comparatively, in our study, a difference of 0.34 to 0.55 t ha^{-1} was observed for semi-dwarfs compared to wild-types, which is larger than previous reports. Meanwhile Robbins (2009) showed a difference in GY of 0.124 to 0.202 t ha^{-1} of semi-dwarf compared to wild-type in near-isogenic lines.

Few studies have focused solely on comparing GY between semi-dwarf haplotypes. Butler et al. (2005) studied a spring wheat population of 140 recombinant inbred lines coming

from a cross between 'Kauz' and 'MTRWA116' in three types of moisture conditions. Their results showed *Rht-B1* semi-dwarf lines to have 0.43 t ha^{-1} higher GY compared to *Rht-D1* semi-dwarfs under full irrigation, with no significant difference under partially irrigated and rain-fed conditions. This is in contrast to our study, which showed *Rht-D1* semi-dwarf lines to be 0.21 t ha^{-1} higher yielding compared to *Rht-B1* semi-dwarfs. Our results support our hypothesis that semi-dwarfs yield higher and among semi-dwarfs, and *Rht-D1* semi-dwarfs have higher GY in contrast to *Rht-B1* semi-dwarfs.

Impact of *Rht* Loci on plant height

As expected, the *Rht* loci significantly affected PH, with *Rht-B1* and *Rht-D1* semi-dwarfs 5.07% and 7.18% shorter in contrast to wild-type, respectively. The differences in PH from this study are less than previous reports, keeping in mind that the wild-type occurred at a very low frequency in the experiment. Compared to wild-type, Robbins (2009) found a 20.5% and 22.4% reduction in PH due to *Rht-B1* and *Rht-D1*, respectively. Other studies have shown similar results (Blake et al. 2009; Flintham et al. 1997; Gale and Youssefian 1985; Trethowan et al. 2002). Overall, PH was negatively correlated with GY ($r = -0.30$ and $P \leq 0.01$), TW ($r = 0.19$ and $P \leq 0.05$), TKW ($r = 0.24$ and $P \leq 0.05$), and KNS ($r = -0.31$ and $P \leq 0.01$), indicating that a shorter stature is favorable for higher GY. This is in agreement with previous studies (Rebetzke and Richards 2000).

Impact of *Rht* loci on yield components

GY is the product of several yield components and previous studies have compared the effect of *Rht* loci on GY components. Using spring wheat, Miralles and Slafer (1995), showed semi-dwarf

lines to have a greater number of kernels m^{-2} compared to wild-types due to higher KNS and spikes m^{-2} in the semi-dwarfs. Kertesz et al. (1991) showed semi-dwarfing to result in a 10% increase in KNS and 13% increase in kernel weight in eastern European environments. Rebetzke and Richards (2000) showed a linear correlation between semi-dwarfing in wheat and kernel number and harvest index. In the current study, all yield components with the exception of SD were significantly affected by the *Rht* loci, with *Rht-D1* semi-dwarfs having a significantly higher TKW and KWS compared to *Rht-B1* semi-dwarfs.

Mapping additional loci for GY, DTH, GY Components, and PH

Until recently, no reports of QTL for GY has been reported in U.S SRWW (Addison et al. 2016). Addison et al. (2016) reported 42 QTL for GY, TW, DTH, and PH with R^2 values ranging from 0.017 to 0.290. Eleven of reported QTL were for GY only and the QTL having the highest R^2 was discovered on chromosome 5B. In our study, no GY associated QTL were found on chromosome 5B. Instead, *QYld.ua-3B* was identified on chromosome 3B with R^2 values of 0.10 to 0.12. Addison et al. (2016) also reported eight QTL for TW with QTL having the highest R^2 of 0.071 being on Chromosome 5D.

Börner et al. (2002) performed an extensive study covering QTL analysis for 20 morphological, agronomical, and disease resistance. They found two and three major joint loci for yield components such as KNS and TKW, respectively. Major QTLs for KNS were found on chromosomes 2DS and 4DL. Major QTLs for TKW were mapped on chromosomes 3AS, 5AL, and 6BS. QTLs for TKW were found on 2DS, 4AL, and 6BL. Comparatively, in this study a major stable region associated with KWS and KNS was found on chromosome 4A with co-localizing *Qkws.ua-4A* and *QKns.ua-4A*. No stable QTL was found for TKW or SD. Börner et al.

(2002) also found four QTL for PH on chromosomes 1AS, 2DS, 4AL and 6AS. In our study, we were able to identify one major genomic region apart from *Rht-B1* and *Rht-D1* being associated with PH. This region contained four QTls with overlapping cM distances. *QPh.ua-3Ba-d* were discovered on chromosome 3B explaining 19% of the variation in the phenotypic data.

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Tables:

Table 1. Summary for traits measured for the Neuse, Bess and the DH[§] lines across 5 site-years[¶].

Traits	Lines	Fay15	Fay16	Fay17	Npt16	Npt17	Mean
Grain yield (t ha^{-1})	Neuse	4.6	5.4	3.7	3.1	2.8	3.8
	Bess	6.0	5.8	4.4	3.4	3.6	4.4
	DH	3.8	5.5	3.7	2.3	3.2	3.8
	Range	1.0-6.4	2.4-7.4	1.3-5.7	0.1-4.4	0.6-5.9	0.1-7.5
Test weight (kg hl^{-1})	Neuse	78.1	79.6	71.6	76.6	72.4	75.3
	Bess	77.5	77.7	70.8	75.7	71.2	73.9
	DH	72.3	78.3	67.3	72.9	69.2	72.4
	Range	67.0-81.9	70.1-84.7	35.1-77.2	61.9-79.7	57.0-80.5	35.1-84.8
Plant height (cm)	Neuse	80.4	83.3	89.0	75.2	91.5	84.4
	Bess	83.0	89.7	92.8	76.1	94.0	88.2
	DH	83.0	85.6	87.9	72.1	87.4	82.9
	Range	66.0-94.0	66.0-111.8	67.3-111.1	50.8-94.00	67.3-111.1	50.8-111.8
Days to heading (days)	Neuse	115.3	.	93.7	.	.	98.4
	Bess	113.0	.	94.9	.	.	98.5
	DH	98.5	.	93.2	.	.	100.0
	Range	91-121	.	82-101	.	.	82-121
Thousand kernel weight (g)	Neuse	.	.	34.9	.	29.5	32.2
	Bess	.	.	33.3	.	27.6	30.5
	DH	.	.	70.1	.	29.8	50.0
	Range	.	.	15.5-61.0	.	16.5-37.1	15.5-61.0
Kernel number $spike^{-1}$	Neuse		.	24.8	.	29.9	27.4
	Bess	.	.	32.4	.	42.1	37.3
	DH	.	.	26.2	.	33.1	29.7
	Range	.	.	13.6-41.8	.	16.1-56.8	13.6-56.8
Kernel weight $spike^{-1}$ (g)	Neuse	.	.	0.9	.	0.9	0.9
	Bess	.	.	1.1	.	1.2	1.2
	DH	.	.	0.8	.	1.0	0.9
	Range	.	.	0.52-1.2	.	0.42-1.5	0.42-1.5
Spike density (spike m^{-2})	Neuse	.	.	17.9	.	22.2	20.1
	Bess	.	.	24.1	.	31.2	27.6
	DH	.	.	71.3	.	331.0	201.1
	Range	.	.	203.0-779.1	.	94.0-539	94.0-779.1

§DH= Doubled haploid

¶Fay15=Fayetteville 2014-15, Fay16=Fayetteville 2015-16, Fay17=Fayetteville 2016-17, Npt16=Newport 2015-2016, Npt17=Newport 2016-17.

Table 2. Analysis of Variance for *Rht*, location and their interaction and narrow sense heritability estimates for traits measured in the doubled haploid population.

Trait	h^2	Mean squares			
		Rht	Location	*Rht* x Location	Rep(Location)
GY	0.82	6.58***	68.66***	0.49	24.53***
TW	0.77	60.89**	869.69***	18.94	420.84***
DTH	0.41	12.46**	6677.00***	21.65	91.70
PH	0.94	832.66***	1922.81***	51.10	19.87
TKW	0.69	58.83***	157.99***	26.95	266.65***
KWS	0.60	0.15***	0.53***	0.01	0.29***
KNS	0.81	139.26***	1162.59***	8.28	860.73***
SD	0.63	13443	213276***	2623.27	538630***

**Significant at P≤0.01 level
***Significant at P≤0.001 level
GY=grain yield, TW=test weight, DTH=days to heading, PH=plant height, TKW-thousand kernel weight, KWS=kernel weight spike-1, KNS=kernel number spike-1, SD=spike density.
ANOVA was calculated using Proc MIXED in SAS.

Table 3. Pearson's correlation for agronomic traits of the DH lines of Neuse x Bess across five site-years.

	GY	TW	DTH	PH	TKW	KNS	KWS	SD
GY								
TW	0.37***							
DTH	0.20*	-0.22*						
PH	-0.30**	0.19*	-0.16					
TKW	0.09	0.40***	-0.28**	0.25*				
KNS	0.63***	0.03	0.26**	-0.31**	-0.31**			
KWS	0.67***	0.29**	0.07	-0.14	0.35***	0.77***		
SD	0.60***	0.36***	0.05	-0.20*	-0.00	0.05	0.02	

*=significant at 0.05 level

**=significant at 0.01 level

***=significant at 0.001 level

GY= grain yield
TW= test weight
DTH= days to heading
PH= plant height
TKW= thousand kernel weight
KNS= kernel number spike^{-1}
KWS= kernel weight spike^{-1}
SD= spike density

Table 4. Differences of Least Squares Means for plant height using combined analysis for *Rht* loci in SAS.

Comparison		Estimate	Standard Error	P-value
Double-dwarf	*Rht-B1* semi-dwarf	4.2668	1.0870	<.0001
Double-dwarf	*Rht-D1* semi-dwarf	6.2136	1.0555	<.0001
Double-dwarf	Wild-type	6.9301	2.1913	0.0016
Rht-B1 semi-dwarf	*Rht-D1* semi-dwarf	1.9468	0.6122	0.0015
Rht-B1 semi-dwarf	Wild-type	2.6633	2.0156	0.1868
Rht-D1 semi-dwarf	Wild-type	0.7165	1.9988	0.7201

Table 5. Differences of Least Squares Means for grain yield using combined analysis for *Rht* loci in SAS.

Comparison		Estimate	Standard Error	P-value
Double-dwarf	*Rht-B1* semi-dwarf	-0.3383	0.1058	0.0014
Double-dwarf	*Rht-D1* semi-dwarf	-0.5405	0.1028	<.0001
Double-dwarf	Wild-type	-0.4158	0.2134	0.0517
Rht-B1 semi-dwarf	*Rht-D1* semi-dwarf	-0.2022	0.05948	0.0007
Rht-B1 semi-dwarf	Wild-type	-0.07746	0.1962	0.6931
Rht-D1 semi-dwarf	Wild-type	0.1247	0.1946	0.5218

Table 6. Differences of Least Squares Means for test weight using combined analysis for *Rht* loci in SAS.

Comparison		Estimate	Standard Error	P-value
Double-dwarf	*Rht-B1* semi-dwarf	0.8196	0.5381	0.1282
Double-dwarf	*Rht-D1* semi-dwarf	1.5937	0.5235	0.0024
Double-dwarf	Wild-type	1.2545	1.0543	0.2345
Rht-B1 semi-dwarf	*Rht-D1* semi-dwarf	0.7741	0.2932	0.0085
Rht-B1 semi-dwarf	Wild-type	0.4349	0.9611	0.6510
Rht-D1 semi-dwarf	Wild-type	-0.3392	0.9528	0.7219

Table 7. Differences of Least Squares Means for days to heading using combined analysis for *Rht* loci in SAS.

Comparison		Estimate	Standard Error	P-value
Double-dwarf	*Rht-B1* semi-dwarf	-4.3161	1.1698	0.0003
Double-dwarf	*Rht-D1* semi-dwarf	-4.3275	1.1367	0.0002
Double-dwarf	Wild-type	-3.1875	2.3599	0.1779
Rht-B1 semi-dwarf	*Rht-D1* semi-dwarf	-0.01143	0.6579	0.9862
Rht-B1 semi-dwarf	Wild-type	1.1286	2.1703	0.6035
Rht-D1 semi-dwarf	Wild-type	1.1400	2.1526	0.5968

Table 8. Differences of Least Squares Means for thousand kernel weight using combined analysis for *Rht* loci in SAS.

Comparison		Estimate	Standard Error	P-value
Double-dwarf	*Rht-B1* semi-dwarf	1.0217	0.6286	0.1049
Double-dwarf	*Rht-D1* semi-dwarf	0.001400	0.6108	0.9982
Double-dwarf	Wild-type	-2.9200	1.2682	0.0219
Rht-B1 semi-dwarf	*Rht-D1* semi-dwarf	-1.0203	0.3535	0.0041
Rht-B1 semi-dwarf	Wild-type	-3.9418	1.1663	0.0008
Rht-D1 semi-dwarf	Wild-type	-2.9214	1.1568	0.0120

Table 9. Differences of Least Squares Means for kernel number spike^{-1} using combined analysis for *Rht* loci in SAS.

Comparison		Estimate	Standard Error	P-value
Double-dwarf	*Rht-B1* semi-dwarf	-3.6415	0.8636	<.0001
Double-dwarf	*Rht-D1* semi-dwarf	-3.8483	0.8392	<.0001
Double-dwarf	Wild-type	-3.3275	1.7422	0.0569
Rht-B1 semi-dwarf	*Rht-D1* semi-dwarf	-0.2068	0.4857	0.6706
Rht-B1 semi-dwarf	Wild-type	0.3140	1.6022	0.8447
Rht-D1 semi-dwarf	Wild-type	0.5208	1.5891	0.7433

Table 10. Differences of Least Squares Means for kernel weight spike^{-1} using combined analysis for *Rht* loci in SAS.

Comparison		Estimate	Standard Error	P-value
Double-dwarf	*Rht-B1* semi-dwarf	-0.08208	0.03041	0.0073
Double-dwarf	*Rht-D1* semi-dwarf	-0.1157	0.02955	0.0001
Double-dwarf	Wild-type	-0.1834	0.06135	0.0030
Rht-B1 semi-dwarf	*Rht-D1* semi-dwarf	-0.03366	0.01710	0.0498
Rht-B1 semi-dwarf	Wild-type	-0.1014	0.05642	0.0732
Rht-D1 semi-dwarf	Wild-type	-0.06770	0.05596	0.2271

Table 11. Differences of Least Squares Means for spike density using combined analysis for *Rht* loci in SAS.

Comparison		Estimate	Standard Error	P-value
Double-dwarf	*Rht-B1* semi-dwarf	12.5533	14.0034	0.3706
Double-dwarf	*Rht-D1* semi-dwarf	1.2113	13.6070	0.9291
Double-dwarf	Wild-type	65.9381	28.2501	0.0201
Rht-B1 semi-dwarf	*Rht-D1* semi-dwarf	-11.3419	7.8754	0.1507
Rht-B1 semi-dwarf	Wild-type	53.3849	25.9795	0.0406
Rht-D1 semi-dwarf	Wild-type	64.7268	25.7680	0.0124

Table 12. Summary of QTL detected for Neuse X Bess double haploid lines across five site-years.

Trait	Marker	Position (cM)	Max LOD	R^2	Additive effect	Source[a]	Site-years
Grain yield							
QYld.ua-3Ba	*IWB7760*	7.9	4.46	0.12	0.31	Neuse	Fay15
QYld.ua-3Bb	*IWB56124*	29.9	2.99	0.11	0.21	Bess	Npt16
QYld.ua-3Bc	*IWB35069*	18.5	3.23	0.10	0.19	Bess	Fay17
Test weight							
QTw.ua-3A	*IWB5723*	41.3	5.49	0.23	1.11	Bess	Fay15, Fay16
QTw.ua-6A	*IWB62193*	11.3	5.46	0.21	1.03	Neuse	Fay15, Npt17
Plant height							
QPh.ua-3Ba-d	*IWB72294*	15.0	5.92	0.19	2.94	Bess	Npt17, Fay17
Days to heading							
QDth.ua-3B	*IWB47459*	114.1	4.42	0.19	1.85	Bess	Fay15, Fay17
QDth.ua-6B	*IWB8078*	8.3	4.27	0.18	1.55	Bess	Fay15, Fay17
QDth.ua-7B	*IWB10879*	5	3.23	0.16	1.46	Neuse	Fay15, Fay17
GY components							
QKws.ua-4A	*IWB71809*	94.2	4.69	0.13	1.07	Bess	Fay17
QKns.ua-4A	*IWB36777*	87.3	3.75	0.08	1.25	Bess	Fay17

Figures:

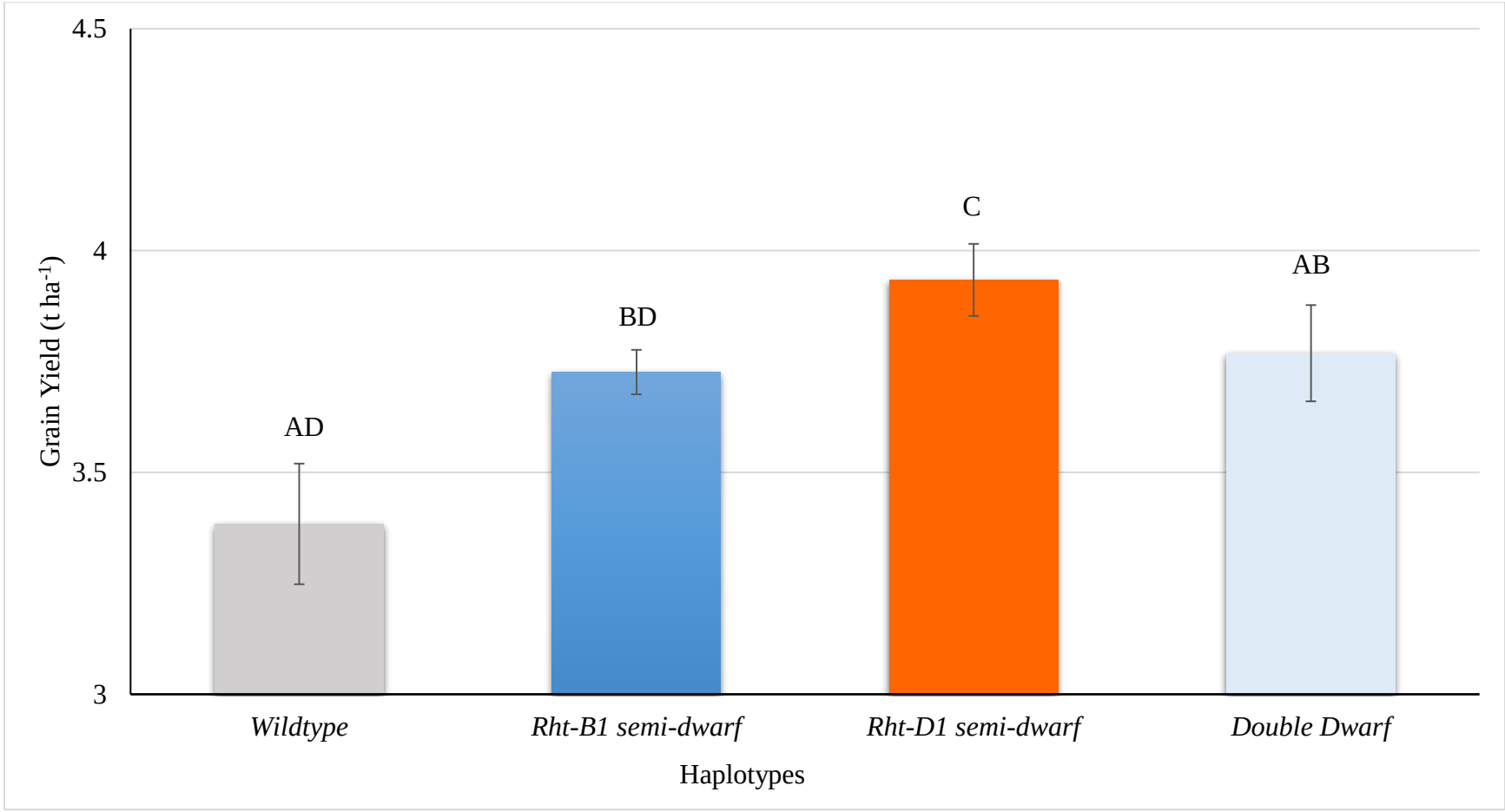


Figure 1: Effect of *Rht* loci on GY across all site-years. Each bar represents one of the four haplotypes with GY on the y axis. Different letter= significantly different at P≤0.05.

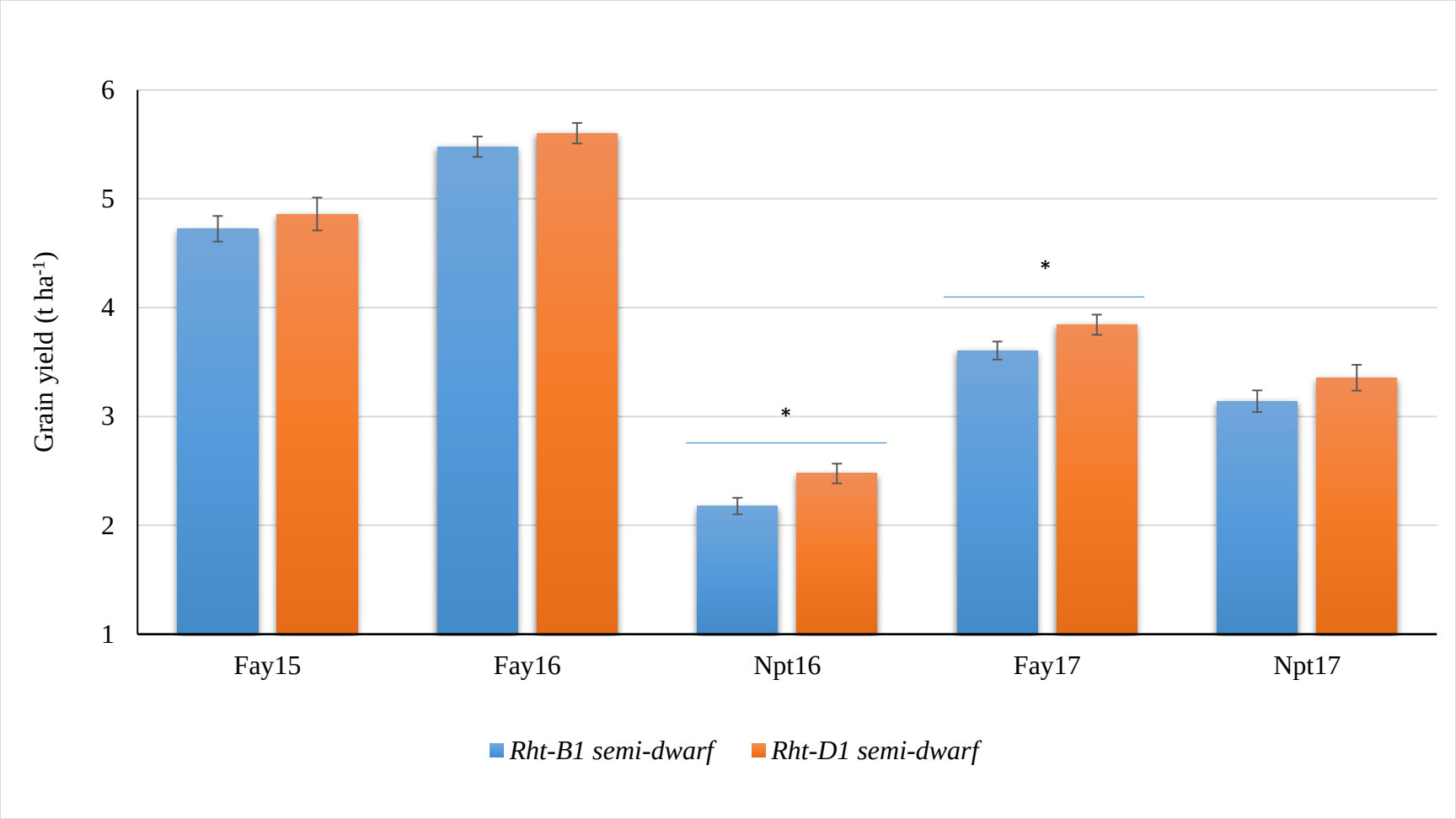
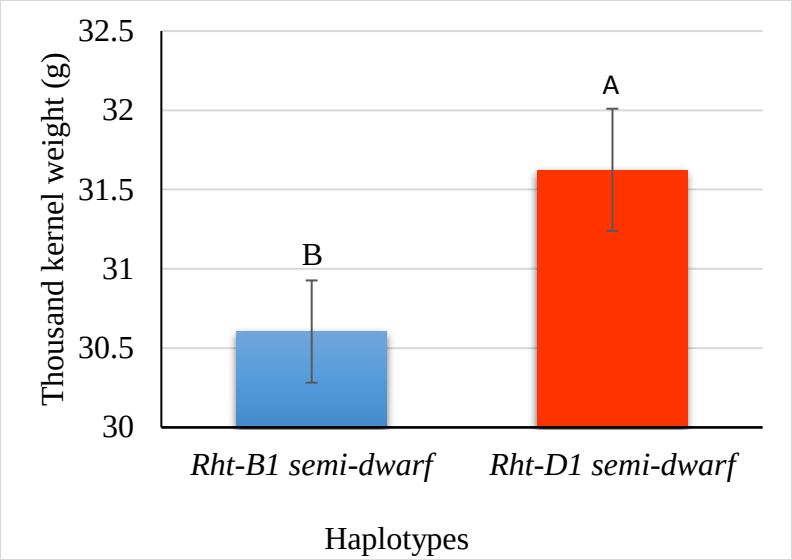
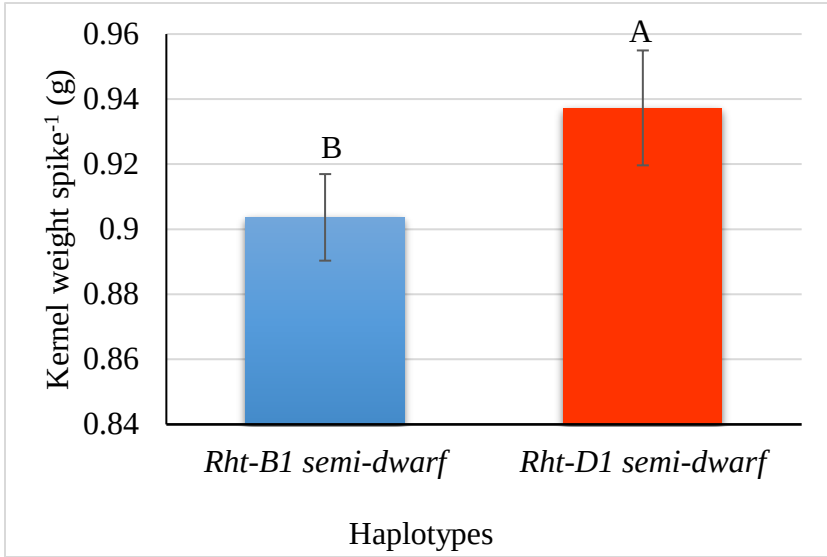


Figure 2. Effect of two *Rht* loci haplotypes on GY of DH population in each of five site-years. Each pair of bar graph represents a site-year. Two haplotypes (*Rht-B1* and *Rht-D1* semi-dwarfing) are compared at each site-year. *Significant at P≤0.05 level

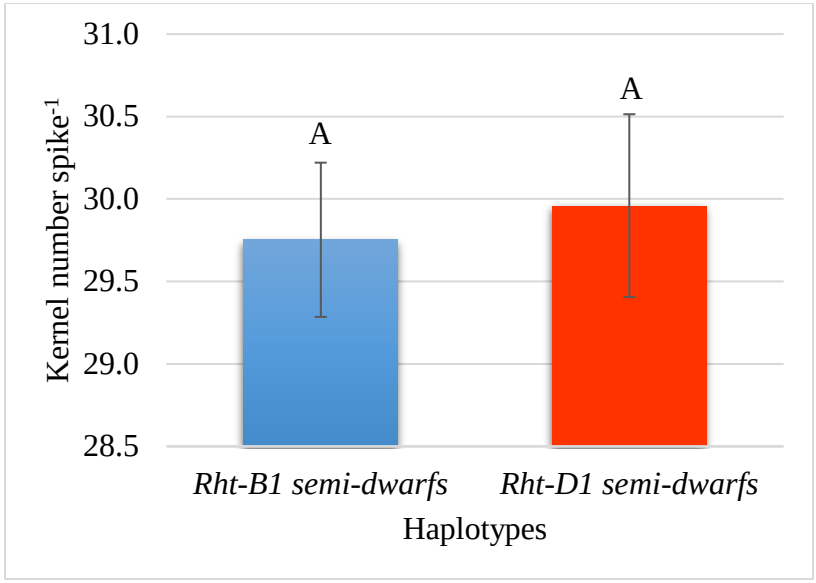


a.

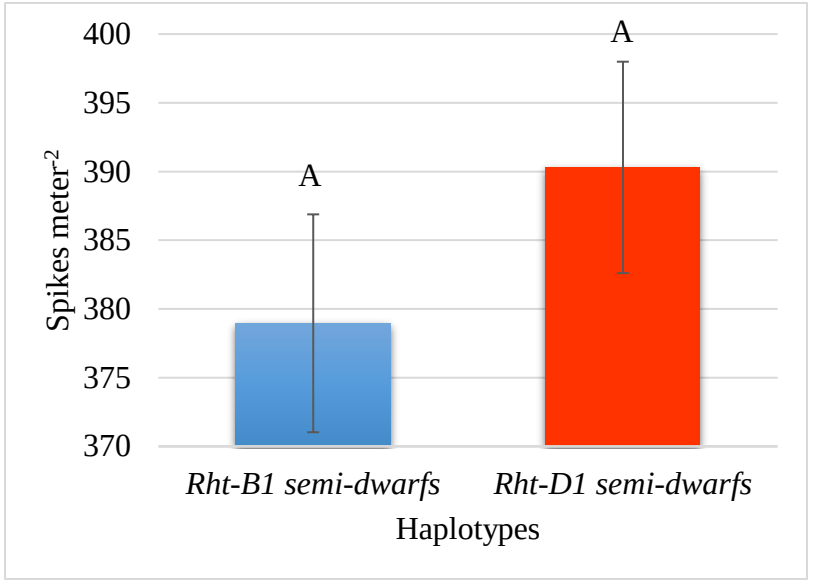


b.

Figure 3. Effect of *Rht* genes on yield components: a. The effect of two haplotypes are show on thousand kernel weight. b. The effect of *Rht-B1* and *Rht-D1* semi-dwarfs are shown on kernel weight spike^{-1}. Different letter = significantly different at P≤0.05.



a.



b.

Figure 4. Effect of *Rht* genes on yield components: a. the effect of two haplotypes are kernel number spike^{-1}. b. The effect of *Rht-B1* and *Rht-D1* semi-dwarfs are shown on spike density. Different letter = significantly different at P≤0.05.

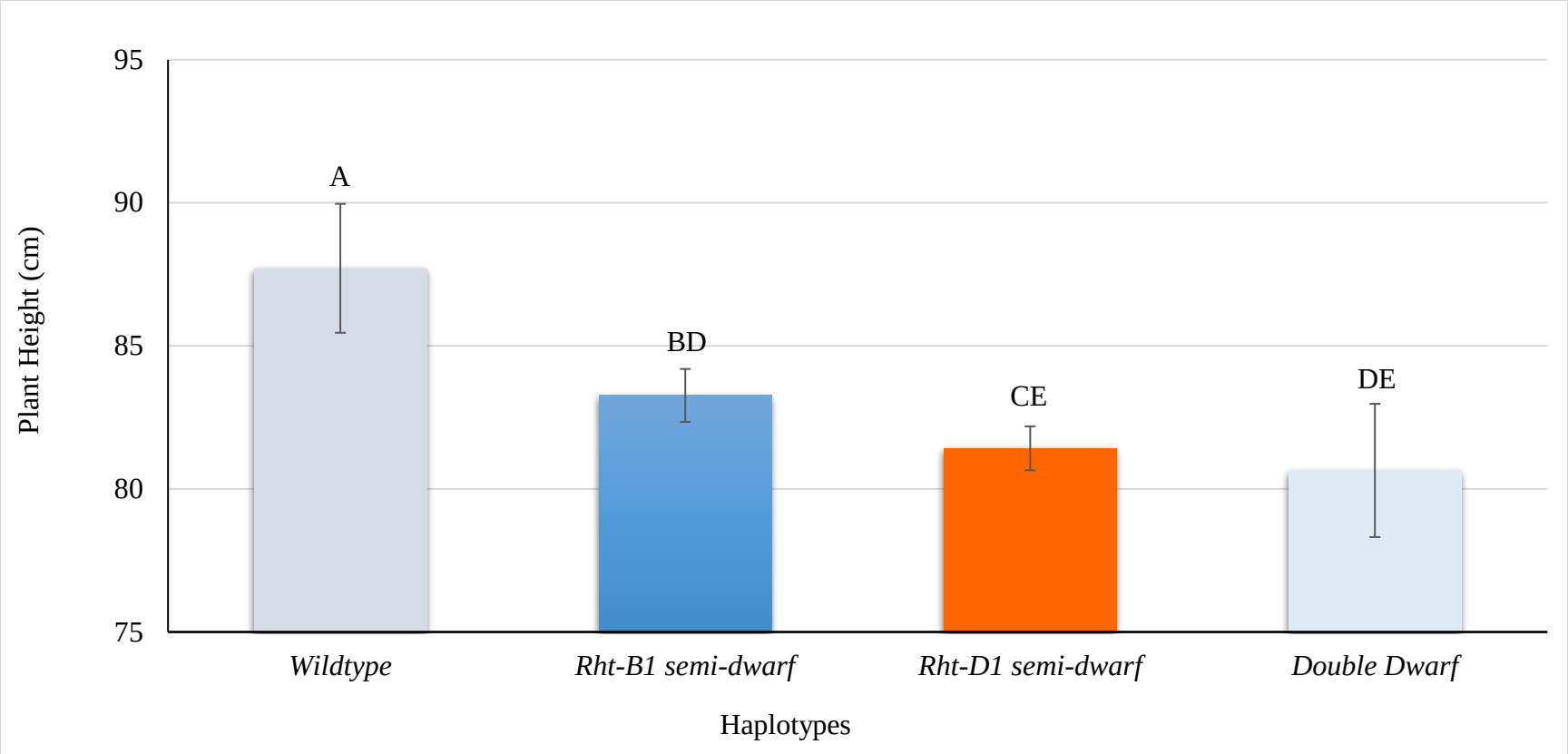


Figure 5. Effect of *Rht* loci on PH. Each of the bars represents one of the four haplotypes with PH in cm on the y axis. Different letter= significantly different at P≤0.05.

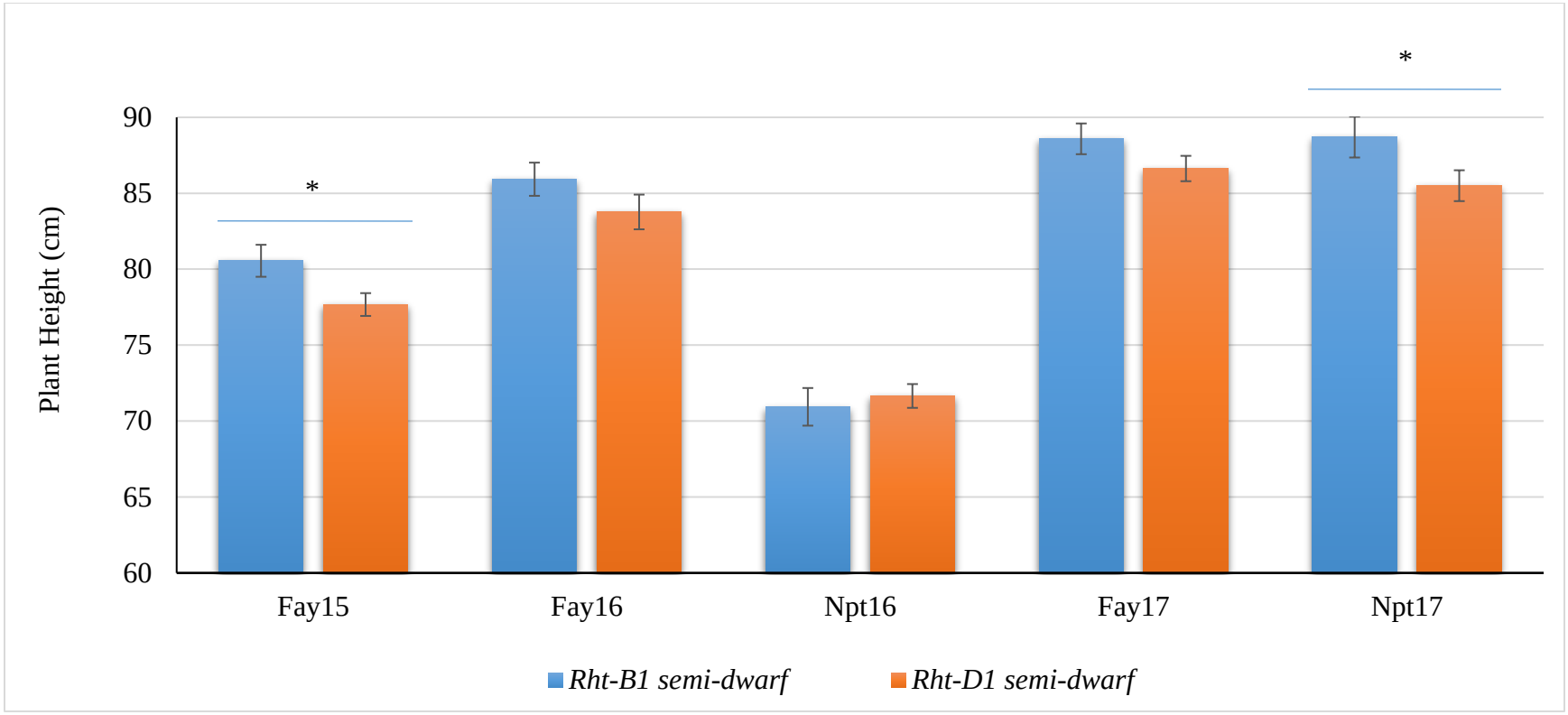


Figure 6. Effect of *Rht* genes on PH in cm. Each pair of the bar graphs represent each of the five site-years. Two haplotypes (*Rht-B1* and *Rht-D1* semi-dwarfing) are compared to each other. *Significant at P≤0.05.

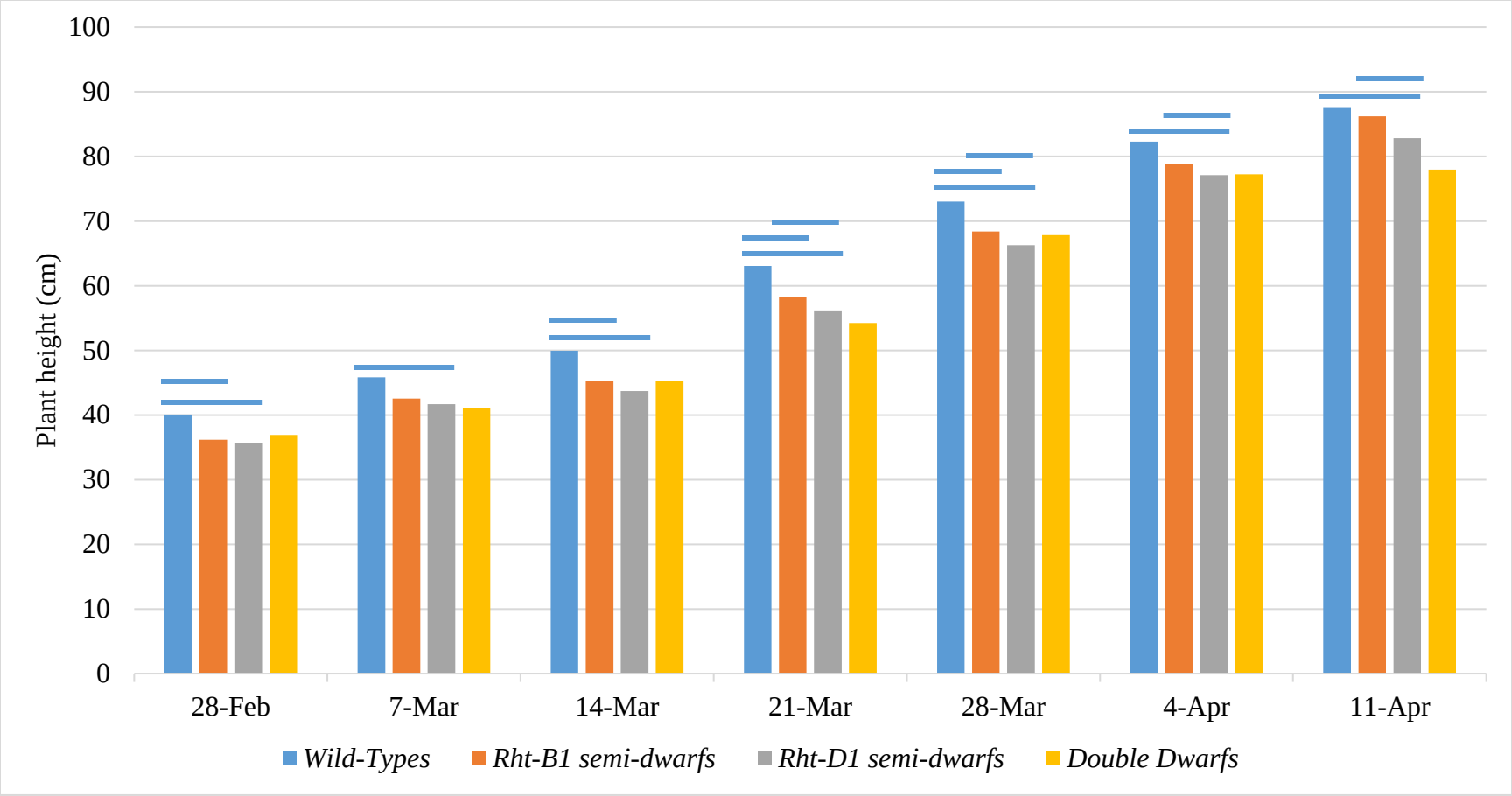


Figure 7. Population growth starting at Feekes 4 stage until full maturity. The bar chart shows growth of four haplotypes measured in centimeters during the growing season. Each measurement was exactly one week apart. Vertical blue bar=significantly different at $P \leq 0.05$

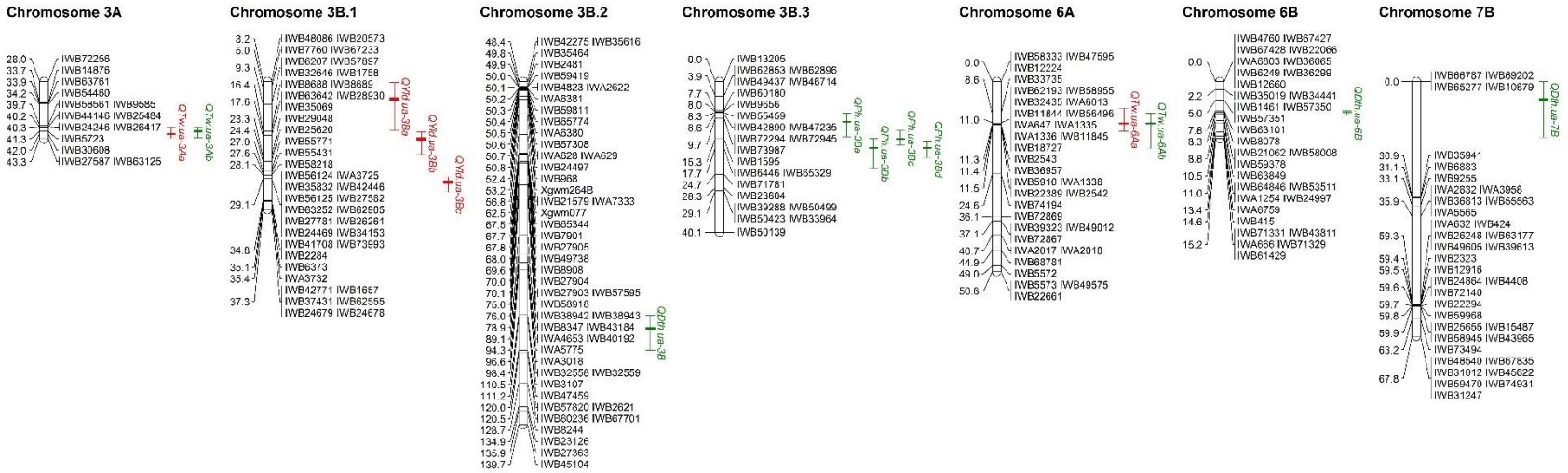


Figure 8. QTL identified for the Bess x Neuse bi-parental mapping population. Chromosome and linkage groups are grouped together. LOD intervals are presented with favorable alleles from Bess in red and favorable alleles from Neuse presented in green. Chromosome 3 is presented in three linkage groups.

Chapter III

Overall Conclusions

Overall Conclusions

Wheat is widely grown in southeastern U.S. Varieties grown are mostly one of the semi-dwarfs, *Rht-B1* or *Rht-D1*. Arkansas was divided between northern state favoring *Rht-B1* semi-dwarfs and southern and southeastern states primarily growing *Rht-D1* semi-dwarfs. The aim of this study was to determine the effect of allelic variation in Green Revolution reduced height loci on GY, PH, and yield components TKW, KWS, and KNS using a doubled haploid population segregating at these loci.

Analysis of variance showed *Rht* to significantly affect PH. The double-dwarfs were to found to be shortest compared to both semi-dwarfs and wild-type. *Rht* significantly affected GY as well. *Rht-D1* semi-dwarfs yielded the highest among the haplotypes (3.9 t ha^{-1}) in all of the site-years compared to double-dwarfs (3.76 t ha^{-1}), *Rht-B1* semi-dwarfs (3.72 t ha^{-1}), and wild-types (3.38 t ha^{-1}) with significant results in Npt16 and Fay17. We observed a similar trend for PH across locations. *Rht-D1* semi-dwarfs were shorter in stature than *Rht-B1* semi-dwarf in all of the site-years except in Npt16. Pleiotropic effect of *Rht* loci on GY were confirmed. *Rht-D1* semi-dwarfs were also found to be higher yield than comparing haplotypes across environment with significant difference observed in Npt16 and Fay17 which supports our hypothesis that *Rht-D1* semi-dwarfing are higher yielding. This can also be seen with yield components, TKW and KWS, where *Rht-D1* semi-dwarfs saw significant increases compared to *Rht-B1* semi-dwarfs. No significant interactions were observed for *Rht* and any of the traits at any of the site-years. GY was found to have a negative correlation with PH and PH was found to have a negative correlation with all other traits except TW and TKW. Weekly measurements of PH was taken for Fay17 site-year in which significant differences were observed between wild-types and both semi-dwarfs for 4 or the seven measurements.

QTL mapping revealed seven region of the genome associated with GY, TW, DTH, PH, KWS, and KNS. GY associated cluster of three closely linked loci were mapped on chromosome 3B.1. This region explained 10 to 12% of the phenotypic variation in yield with Bess providing the favorable alleles. TW had two QTL associated with it. They were *QTw.ua-3A* and *QTw.ua-6A* and explained up to 23% and 21% of the phenotypic variation, respectively. Three major QTLs were identified for DTH on chromosomes 3B.2, 6B, and 7B which explained up to 19% of the phenotypic variation with Neuse as the favorable allele source. Major QTLs associated with KWS and KNS were located on chromosome 4A explaining up to 14% of the phenotypic variation. In addition to major *Rht-B1* and *Rht-D1* loci, a short region of the chromosome 3B.2, containing four major QTL, was found to be associated with PH and explained up to 19% of the phenotypic variation.

Considering the agronomic section of this study focusing on results for *Rht* loci comparison, we can conclude that *Rht-D1* semi-dwarfs are optimum and favorable for GY and PH in Arkansas environment compared to *Rht-B1* semi-dwarfs. We suggest future breeding efforts should focus on developing lines with *Rht-D1* semi-dwarfing. We also found potential QTL for most of the agronomic traits measured. Further investigation of these QTL and their effect on GY and other traits is highly encouraged.