DWARFING GENES IN SPRING WHEAT:

AN AGRONOMIC COMPARISON OF *RHT-B1*, *RHT-D1*, AND *RHT8*

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Plant Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

November 2009

APPROVAL

of a thesis submitted by

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the Division of Graduate Education.

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Amber Marie Robbins

November 2009

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NOMENCLATURE

Rht-B1a	Formerly *rht1*. Exhibits wild type stature.
Rht-B1b	Formerly *Rht1*. Exhibits semi-dwarf stature.
Rht-D1a	Formerly *rht2*. Exhibits wild type stature.
Rht-D1b	Formerly *Rht2*. Exhibits semi-dwarf stature.
rht8	Exhibits wild type stature.
Rht8	Exhibits semi-dwarf stature.
Ppd-D1	Exhibits photoperiod-insensitivity.
ppd-D1	Exhibits photoperiod-sensitivity.

ABSTRACT

Rht-B1b and *Rht-D1b* (formerly *Rht1* and *Rht2*) dwarfing genes have been used extensively since the Green Revolution to reduce height and increase yield in hexaploid wheat (*Triticum aestivum* L.). They have been used in the development of hundreds of modern cultivars. Semi-dwarf wheat varieties containing these GA-insensitive dwarfing genes generally yield more grain than their tall *Rht-B1a* and *Rht-D1a* (formerly *rht1* and *rht2*) counterparts. However, *Rht-B1b* and *Rht-D1b* are better adapted to high-input environments and can reduce plant performance under adverse conditions such as heat or drought stress. In such environments, *Rht8*, a GA-sensitive dwarfing gene, could prove beneficial.

This study aimed to assess agronomic performance of *Rht8* compared to *Rht-B1b* and *Rht-D1b* using near-isogenic lines of the six possible genotypes (*Rht-B1b, Rht-B1a, Rht-D1b, Rht-D1a, Rht8,* and *rht8*) in four different spring wheat backgrounds. Field trials were conducted during the 2008 and 2009 growing seasons in Bozeman and Kalispell, MT. Traits measured included plant height, coleoptile length, stem solidness, days to heading, yield, flag leaf characteristics, harvest index, and others. Measurements were taken from field trials except coleoptile length, which was grown in a temperature and light controlled growth chamber.

Rht genotype had a significant effect on plant height, coleoptile length, and yield. Height reductions of 20.5%, 22.4%, and 10.1% were observed for *Rht-B1b, Rht-D1b,* and *Rht8*, respectively. *Rht8* showed no significant effect on coleoptile length, while *Rht-B1b* and *Rht-D1b* exhibited reductions in coleoptile length of 21.3% and 22.8%. Rht-B1b and Rht-D1b both showed a 6.1% and 14.1%. increase in yield potential. In contrast, Rht8 showed a yield decrease of 5.3%.

Of most interest to plant breeders are those traits that effect yield. Yield is a complex, multi-genic trait that is affected by many plant characteristics and environmental factors. Because of the quantitative nature of yield, it is impacted by not only the presence of dwarfing genes, but also genetic background and environmental variables.

This study suggests that *Rht-B1b* and *Rht-D1b* are superior, in terms of yield potential, to *Rht8.* However, additional evaluation of *Rht8* in differing environments is needed to elucidate its precise effects on yield and other agronomic traits.

CHAPTER 1

INTRODUCTION

Reduced height in cereals is often associated with increases in yield due to a reduced risk of lodging, increase in partitioning of assimilates to the grain (Evans, 1993), and more fertile florets per spikelet (Brooking and Kirby, 1981).

A reduction in height is usually caused by reduced height trait genes (Rht). Rht dwarfing genes are broadly characterized depending on their response to gibberellic acid (GA), a classic plant hormone that affects many aspects of plant growth and development, including seed germination, floral induction, fruit set, and plant height. Rht mutants are either insensitive (show no response to exogenous GA) or sensitive.

The *Rht-B1b* and *Rht-D1b* genes, formerly known as *Rht1* and *Rht2*, respectively, are the most common dwarfing genes in hexaploid wheat (*Triticum aestivum* L.). They have been used in the development of hundreds of wheat cultivars to enhance plant performance since their introduction during the Green Revolution of the 1960's and, as of the early 21st century, were found in over 95% of released cultivars in developing countries (Heisey et al., 1999).

Semi-dwarf wheat varieties containing these dwarfing genes generally yield more grain than their tall *Rht-B1a* and *Rht-D1a* (formerly *rht1* and *rht2*) counterparts. However, these dwarfing genes are not beneficial in all environments. Plants with a shorter stature are better adapted to irrigated, high-input environments, while taller plants are considered to have better yield stability under adverse conditions, such as heat and/or drought stress (Reynolds et al., 1994). In areas where high summer temperatures and

drought frequently occur, *Rht-B1b* and *Rht-D1b* can reduce, rather than improve, plant performance (Korzun et al., 1998). In such environments, a different source of semi-dwarfism is favored- *Rht8* from the Japanese variety Akakomugi (Worland and Law, 1986).

Rht8 is widespread in varieties grown in South and Central Europe (Borojevic and Borojevic, 2005). Recently, Australian wheat breeding programs have shown renewed interest in *Rht8* for its ability to reduce plant height without reducing coleoptile length or early vigor (Rebetzke et al., 1999). The Australian wheat growing regions have temperate and grassland climates like much of Montana and the Northern Great Plains (Australia, 2009; United States, 2009), making *Rht8* of interest for wheat breeding programs in Montana as well.

This study focuses on assessing not only the effect of *Rht8* on several agronomic traits but also comparing *Rht8* to *Rht-B1b* and *Rht-D1b*, with height, yield, coleoptile length, and stem solidness being of greatest interest because of their economic importance. Yield is among the most economically important trait and height, coleoptile length, and stem solidness all indirectly affect yield potential.

Near-isogenic lines (NILs) were used to evaluate the effects of the different Rht dwarfing genes. Near-isogenic lines are genetically identical with the exception of the gene(s) of interest and are generated by making an initial cross between two different varieties and then crossing it back to one of the varieties for several generations. NILs are the most precise population available to study the effects of a specific gene(s) when it is

backcrossed into a variety of different backgrounds because they allow assessment of a single gene or group of genes by removing confounding effects of differing backgrounds.

CHAPTER 2

LITERATURE REVIEW

Origin and History of *Rht-B1b* , *Rht-D1b*, and *Rht8*

The GA-insensitive height reducing genes *Rht-B1b* and *Rht-D1b* originated in the 1930's in the dwarf variety Norin 10, a derivative of the Japanese variety Daruma (Allan, 1989). Shortly after World War II, S. C. Salmon, a wheat breeder with the USDA, visited Japan as an advisor to the occupation army. During his visit, he received several wheat samples, and among them was the variety Norin 10, which he sent to the USDA Small Grains Collection Facility. In 1948, Norin 10 was obtained by Orville Vogel, a USDA-ARS wheat breeder in Pullman, WA, who then crossed Norin 10 with the high-yielding variety Brevor 14 (Allan, 1989). The Norin 10 x Brevor 14 cross was then used by Norman Borlaug and others as part of wheat improvement programs in the United States and at the International Maize and Wheat Improvement Center (CIMMYT) (Ellis et al, 2002).

The Japanese variety Akakomugi was the source for most of the European cultivars carrying the *Rht8* dwarfing gene (Worland et al., 1998). *Rht8* is widespread in southern and central European wheats as well as several Russian cultivars (Worland et al., 1998) Akakomugi was first used by the Italian breeder Strampelli in the 1920's to introduce genes not only for semi-dwarfism (*Rht8*) but also, unknowingly, for early maturity (*Ppd-D1*) (Worland and Law, 1986, Korzun et al., 1998).

From Italy, *Rht8* made its way to Argentina before World War II and then to Europe and the former Soviet Union after World War II (Borojevic and Borojevic, 2005). Unlike Rht-*B1b* and *Rht-D1b*, the *Rht8* dwarfing gene from Akakomugi is sensitive to exogenous GA.

Genetics of *Rht-B1b*, *Rht-D1b*, and *Rht8*

Rht-B1 is located on chromosome 4B (Gale and Marshall, 1976), which was confirmed in both hexaploid wheat (Rao, 1980) and durum wheat (Blanco et al., 1998). *Rht-D1* is located on chromosome 4D (Gale et al., 1975).

Traditionally, selection for the *Rht-B1b* and *Rht-D1b* dwarfing genes was determined by testing seedlings with gibberellic acid (GA). Plants with the mutant dwarfing gene show no response to exogenous GA. Both *Rht-B1b* and *Rht-D1b* dwarfing genes are insensitive to exogenous GA. With the introduction of marker-assisted selection, perfect markers for *Rht-B1b* and *Rht-D1b* were developed in a doubled haploid population that was segregating for the *Rht-B1b* and *Rht-D1b* alleles (Ellis et al., 2002). Perfect markers detect the specific base-pair mutation responsible for the semi-dwarfing phenotype.

The mutations involved in the *Rht-B1b* and *Rht-D1b* semi-dwarf phenotypes disrupt the GA signaling pathway. The wild type proteins are thought to act as negative repressors of GA signaling and GA acts by repressing their function (Hussain and Peng, 2003). Peng et al. (1997) identified base substitutions in both *Rht-B1b* and *Rht-D1b.* In both cases, the mutations affects the N-terminal region of their transcribed DELLA

proteins via a substitution which produces a stop codon shortly after the transcription start site (Peng et al., 1997), resulting in their characteristic GA-insensitivity. DELLA proteins are a subfamily of GRAS proteins, which are thought to act as transcriptional regulators (Pysh et al., 1999). *Rht-B1b* and *Rht-D1b* produce stop codons in the DELLA domain, a 27 amino acid motif at the N-terminus, and in their truncated form, these proteins are thought to act as constitutive repressors of GA-mediated growth (Peng et al., 1997).

Gale et al. (1982) found the location of a major *Rht* allele on chromosome 2D with a backcross monosomic analysis using varieties Sava (derived from Italian wheats) and Koga II. This allele accounted for nearly all of the height difference between the two parent varieties. Another study, by Law et al. (1981), determined that the dwarfism of Mara, an Italian variety derived from Akakomugi, was partially caused by chromosome 2D. The causal gene was later designated as *Rht8.* The microsatellite marker WMS 261 is located 0.6cM distally from *Rht8* can be used as a marker to identify lines carrying the Rht8 dwarfing gene (Korzun et al., 1998).

Rht8 has been shown to be closely linked to the photoperiod-insensitivity gene *Ppd-D1* (Korzun et al., 1998). Photoperiod-sensitive wheat varieties require long days for floral induction, while photoperiod-insensitive varieties have the ability to flower independently of photoperiod. Several studies have shown that varieties exhibiting photoperiod-insensitivity also exhibit earlier heading and shorter stature than their photoperiod-sensitive counterparts (Marshall et al., 1989; Blake et al., 2009). In addition to effects on heading date and height, Dyck et al. (2004) found that photoperiod

insensitivity had a significant effect on yield. In the current study, lines were screened for photoperiod-insensitivity and removed to reduce confounding effects.

Agronomic Traits of *Rht-B1b*, *Rht-D1b*, and *Rht8*

Height

The height reduction associated with *Rht-B1b* and *Rht-D1b* arises from GA-insensitivity that causes a decrease in cell elongation in juvenile leaf and stem tissue, which leads to an overall reduction in plant height. Height reductions for cultivars carrying the *Rht-B1b* and *Rht-D1b* dwarfing genes are similar to each other. *Rht-B1b* and *Rht-D1b* were found to reduce plant height by 15% (Gale and Youseffian, 1985) and 24% (Allan, 1986). Another study found a reduction of 14% and 17% for Rht-*B1b* and *Rht-D1b*, respectively (Flintham et al., 1997). Trethowan et al. (2001) reported an average height reduction of 36% in a population of *Rht-B1b* near-isogenic lines. Blake et al. (2009) suggested that final plant height is influenced by not only genotype but also a variety of environmental factors, such as heat, drought, and nutrient deficiencies.

Rht8 has been shown to reduce height by approximately 10% in studies from the UK, Germany, and former Yugoslavia (Worland and Law, 1986; Worland et al., 1998). Reductions of 3.49% (Börner et al., 1993), 7.3% (Rebetzke et al., 1999), and 12.5% (Rebetzke and Richards, 2000) have also been reported.

Yield

Reports of the advantages and disadvantages of different *Rht* alleles and their standard height counterparts have drawn varying conclusions. Increased yield potential for *Rht-B1b* and *Rh-D1b* has been noted under high-input growing conditions (Knott, 1986; Hedden, 2003; McNeal et al., 1972)

Although *Rht-B1b* and *Rht-D1b* dwarfing genes have the potential to increase yield of wheat grown in optimal conditions, these dwarfing genes have been associated with reductions in yield in environments with low-inputs or abiotic stresses (Laing and Fischer, 1977; Anderson and Smith, 1990; Richards, 1992)

The yield advantages of *Rht-B1b* and *Rht-D1b* are less obvious in spring wheat than in winter wheat as well as in conditions of heat or drought stress (Flintham et al., 1996). Heat and drought stress during ear initiation can reduce grain number through a reduction in the number of competent florets and pollen viability and can reduce grain weight as a result of shortened grain-fill period (Hoogendoorn and Gale, 1988).

Rht-B1b, *Rht-D1b*, and possibly *Rht8,* are associated with increased floret fertility which may counteract the negative effects on yield observed with some Rht genes (Gale and Youseffian, 1985). Yield increases in semi-dwarf wheat cultivars are due, in part, to increased partitioning of assimilates into the developing grain rather than into the stem for elongation (Flintham et al., 1997).

Several studies suggest that there is not a significant difference between *Rht-B1b* and *Rht-D1b* in terms of yield improvement. In Montana and Saskatchewan trials, semi-dwarf lines containing *Rht-B1b* or *Rht-D1b* generally yield more than standard height

lines, except in very low yielding environments, where the standard height lines exhibited a yield advantage (Knott, 1986; McNeal et al., 1972).

Yield increases of 24% (Flintham et al., 1997) and 16% (Singh et al., 2001; Allan, 1986) have been reported for *Rht-B1b* and *Rht-D1b*. Yield increases of 21% (Chapman et al., 2007) for *Rht-B1b* and 30% (Blake et al., 2009) and 18% (Chapman et al., 2007) for *Rht-D1b* have been reported. For cultivars carrying *Rht8,* yield increases of 12% (Gale et al., 1982), 9.7% (Rebetzke and Richards, 2000), and 3.8% (Börner et al., 1993) have been reported.

Coleoptile Length

Crop establishment is a major determinant of yield (Paulsen, 1987) and coleoptile length is an important factor in seedling emergence and crop establishment. Reduced coleoptile length is associated with reduced emergence and subsequent poor crop establishment (Allan, 1980). In modern wheat cultivars, one of the most important determinants of coleoptile length is the presence of the Rht semi-dwarfing genes.

Standard height (tall) wheats have long coleoptiles, due to normal cell elongation in the presence of endogenous GA, and *Rht-B1b* and *Rht-D1b* semi-dwarf wheats, which are GA-insensitive, have shortened coleoptiles (Keyes et al., 1989). Allan (1980) found that *Rht-B1b* and *Rht-D1b* reduce coleoptile length in a similar proportion to their reduction in plant height. Wheat cultivars carrying *Rht-B1b* and *Rht-D1b* dwarfing genes have a limited coleoptile length of about 7.0 cm, while the coleoptiles of standard height wheats can reach up to 13.0 cm (Whan, 1976). Another study, by Trethowan et al.

(2001), reported standard height average coleoptile length at 12.4 cm and *Rht-B1b* semi-dwarf average coleoptile length at 7.8 cm.

In cultivars containing the GA-sensitive *Rht8* dwarfing gene, there seems to be a negligible effect on coleoptile length (Konzak, 1987). Additionally, Rebetzke et al. (1999) reported *Rht8* semi-dwarf coleoptiles as long as those of the standard height parent.

Stem Solidness

The wheat stem sawfly, *Cephus cinctus* Norton, is a major insect pest of wheat and other cereals across areas of western North America as well as Canada (Davis, 1955). Adults deposit eggs in the stems and when the larva hatch, they feed on the parenchyma and vascular tissue inside of the stem, moving down the stem until they reach near ground level, where they cut around the inside of the stem. Then, they move down into the remaining portion of the stem to pupate and overwinter (Hayat, 1993). Agricultural losses caused by wheat stem sawfly are estimated at $25 million per year (Montana State University, 1997). Currently, the main control method has been the use of solid stemmed cultivars.

Stem solidness is caused by the development of undifferentiated parenchymous cells, or pith, inside the stem. The thickness of the parenchymal cell walls has been reported to have a direct relationship with larval mortality in wheats with solid stems (Roemhild, 1954). Thus far, no published studies have examined the effect of Rht dwarfing genes on stem solidness. The current study examines the effect of Rht genotype on stem solidness.

CHAPTER 3

MATERIALS AND METHODS

Population Structure

Near-isogenic lines (NIL) were used to compare the *Rht-B1b, Rht-D1b,* and *Rht8* dwarfing genes. NILs were derived through a series of backcrosses with the recurrent parents being of the varieties Amidon, Fortuna, JC73, Scholar, and Thatcher. The Rht dwarfing gene donors included Hi-Line, McNeal, and Mara, contributing the *Rht-B1b, Rht-D1b,* and *Rht8* dwarfing genes, respectively. (Table 1) Within each recurrent parent group, Rht mutant/wild type pairs were derived from a single F2 heterozygote plant. For each Rht type (e.g. *Rht-B1*, *Rht-D1*, or *Rht8*), two mutant/wild type pairs were included. Backcross information for this study is contained in Appendix C.

Table 1 Recurrent and Donor Parents

	Date of Release	**Origin**	**PI number**
Recurrent Parents			
Amidon	1988	North Dakota	PI 527682
Fortuna	1966	North Dakota	CI 13596
JC73	1969	Burundi, Africa	PI 351872
Scholar	1999	Montana	PI 607557
Thatcher	1948	Minnesota	PI 168659
Donor Parents			
HiLine	1991	Montana	PI 549275
McNeal	1994	Montana	PI 574642
Mara	1958	Italy	PI 244854

A basic backcrossing scheme is shown in Figure 1. One variety is the donor of the gene(s) of interest, referred to as the donor parent, and the other is the recipient, referred to as the recurrent parent. An initial cross is made between then recurrent and donor parents to yield to F1 generation. The F1 progeny, which contains 50% of the genes from each parent, are crossed back to the recurrent parent to yield BC1 progeny that contain 75% of the genes of the recurrent parent and 25% of the donor parent genes. Each successive backcross halves the percent of donor parent genes, i.e. BC2 contains only 12.5% donor genes, BC3 contains 6.25%, and so on. In general, the donor parent was the male and the recurrent parent was the female. Selection for the gene(s) of interest (from the donor parent) occurs in every generation. After the final backcross, F2 individuals are selected for homozygosity at the gene(s) of interest. These individuals are the NILs used for testing the effect of the selected gene(s).

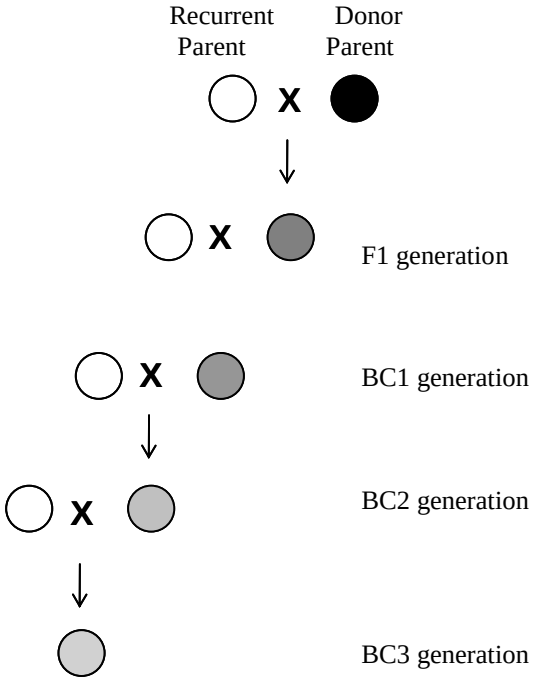

Figure 1 Basic Backcross Scheme

Genotyping of Near-Isogenic Lines

Entries having Hi-Line as the *Rht-B1b* donor were screened for *Rht-B1b* (mutant) and *Rht-B1a* (wild type). Entries having McNeal as the *Rht-D1b* donor were screened for *Rht-D1b* and *Rht-D1a* alleles. Primer sets used to screen for *Rht-B1* and *Rht-D1* have been previously described (Ellis et al., 2002) and polymerase chain reaction conditions followed published protocols. Amplified products were visualized using a 1.2% agarose gel stained with ethidium bromide.

Entries having Mara as the *Rht8* donor were screened with primer set WMS 261 for *Rht8* (mutant) and *rht8* (wild type alleles). WMS 261 yields a 192 bp fragment that is diagnostic for *Rht8*. Polymerase chain reaction conditions followed protocol reported by Ellis et al. (2005). Amplified products were visualized on a 12% acrylamide gel stained with ethidium bromide.

Rht8 has been shown to be linked to photoperiod-insensitivity gene *Ppd-D1*, which can have pleiotropic effects on several agronomic characteristics (Korzun et al., 1998). All recurrent parents were photoperiod sensitive (*ppd-D1).* Entries with Mara as the donor parent were screened for *Ppd-D1* as described by Beales et al. (2007) to identify those entries in which only the *Rht8* allele was introduced. Those entries found to carry *Ppd-D1* (photoperiod-insensitive) were removed from the experiment and replaced with a *ppd-D1* (photoperiod-sensitive) entry. In the case of entries with Thatcher as the recurrent parent, a replacement could not be made, so all Thatcher entries were removed from the experiment.

Experimental Design and Layout

All experiments were planted in a randomized complete block-split plot design with recurrent parent as the main plot. Rht genotypes and a recurrent parent control were the subplots. There were four main plots (one corresponding to each recurrent parent) and thirteen subplots (two mutant/wild type pairs for *Rht-B1*, *Rht-D1*, and *Rht8* plus a recurrent parent control).

Entries were planted in single row plots with a border row on each side. The border varieties were Newana, a semi-dwarf variety, for the mutant lines and the standard height variety Fortuna for the wild type lines.

Field Location, Growing Conditions, and Harvest Conditions

Field trials were conducted at the Arthur H. Post Field Research Farm in Bozeman, MT in 2008 and 2009. Geographical coordinates for the Post Farm are 45° 41' N, 111° 00' W with Amsterdam silt loam soil and elevation of 1454.5 m. In 2008 and 2009, both a dryland and irrigated experiment were planted in Bozeman. In addition, a dryland experiment was planted in Kalispell, MT in 2009 at the MSU Northwestern Agricultural Research Center. Geographical coordinates are 48°15'N, 114°15'W with Creston silt loam and elevation of 886.7 m. Temperature and precipitation for the 2008 and 2009 growing seasons are listed in Table 2.

Table 2 Precipitation and Temperature by Location and Year

Year			April	May	June	July	Aug.	Year Total	Year Ave.
Bozeman, MT	2008	Precipitation (cm)	4.24	8.38	6.76	3.25	1.78	47.02	-
		Temperature (°C)	3.61	10.39	14.44	19.67	19.28	-	6.61
	2009	Precipitation (cm)	7.16	4.09	6.65	7.09	3.84	43.33	-
		Temperature (°C)	5.06	12.11	14.06	18.56	18.39	-	6.84
	Average 1958-2009	Precipitation (cm)	4.14	6.65	6.93	3.48	3.20	40.72	
		Temperature (°C)	5.72	10.76	14.83	18.72	18.11		6.36
Kalispell, MT	2009	Precipitation (cm)	2.49	4.11	5.03	6.20	2.51	48.29	-
		Temperature (°C)	5.42	11.83	15.11	19.50	18.94	-	5.60
	Average 1980-2009	Precipitation (cm)	4.50	6.15	8.03	4.34	2.95	40.72	
		Temperature (°C)	6.22	10.83	14.28	18.00	17.50		6.36

Entries were grown in the greenhouse in early 2008 for seed increase and again in late 2008. In 2008 and 2009, experiments were planted in both a dryland and irrigated setting in Bozeman, MT on May 6th, 2008 and May 14th, 2009. Seeding rates were 2.3 g/m for dryland and 3.3 g/m for irrigated and both were planted in 3 m rows. Irrigation was applied to the irrigated experiment at the boot stage and irrigation conditions were as follows: 5.7 cm applied July 8th, 2008 and 6.4 cm applied July 21st and 24th, 2009. In Bozeman, nitrogen fertilizer was added at 56 kg/ha in 2008 and 2009.

Due to severe hail in Bozeman in 2008, neither the dryland nor irrigated experiments were harvested, thus data for yield, harvest index, test weight, fertile tiller number, and grain protein were not obtained.

In 2009, the Bozeman dryland experiment was cut by hand using a Japanese rice knife on September 2nd, bundled, and left to dry until threshing with a Vogel stationary plot thresher on September 9th. The Bozeman irrigated experiment was cut by hand using a Japanese rice knife on September 8th, bundled, and left to dry until threshing with a Vogel stationary plot thresher on September 14th.

In 2009, a dryland experiment was planted in Kalispell, MT. Rows were 4.6 m and were planted at a seeding rate of 2 g/m on May 22nd, 2009 and fertilized with 162.4 kg/ha of nitrogen and 67.2 kg/ha of potassium on May 20th, 2009. No irrigation was applied. The experiment was harvested by hand with a Japanese rice knife and threshed with a Vogel stationary plot thresher on September 1st, 2009.

Coleoptile Length

Short coleoptiles can lead to reduced emergence and stand establishment (Allen, 1980). To measure coleoptile length, entries were set up in a complete randomized block experiment with four replications and three seeds planted per entry in the Plant Growth Center (Montana State University) in 2008. Seeds were planted into vermiculite in trays, spaced at 2.5 cm apart, and kept in a dark, 20°C growth chamber for twelve days. The trays were watered for the first five days only. After twelve days, three coleoptiles were measured, in cm, for each entry and averaged to give the final measurement.

Heading Date

Heading date for each entry was recorded in the field as the day when 50% of the heads had emerged from the flag leaf sheath. Dates were recorded in Julian days. Heading dates were recorded in Bozeman dryland and irrigated in 2008 and 2009 and Kalispell in 2009.

Stem Solidness

Five stems per plot were pulled at random, near crop maturity. A cross section was cut through the center of each internode and a total of five internode ratings were made using a 1-5 scale where 1 designates a hollow stem and 5 designates a completely solid stem, as shown in Figure 2. Internode scores were summed to give each stem a single score. The single stem scores were averaged and recorded as one final stem solidness score for each entry. Stem solidness was measured in Bozeman dryland and irrigated in 2008 and 2009 and Kalispell in 2009.

Figure 2 Stem Soldiness Scale

Plant Height

Plant heights were found by measuring the height, in centimeters, from soil surface to the estimated average height of two or three main tillers, excluding awns. Two measurements per plot were taken at random and averaged to give a final plant height for each plot. Measurements were taken in 2008 and 2009 in both Bozeman and Kalispell, MT.

Flag Leaf Width, Flag Leaf Length, and Fertile Tillers

Flag leaf width was found by measuring, in cm, across the widest portion of the flag leaf. Two measurements were taken at random per plot and averaged to give a final flag leaf width for each plot. Flag leaf length was found by selecting two leaves at random and measuring their length in cm. As with flag leaf width, two measurements were taken at random per plot and averaged to give a final flag leaf length measurement for each plot. Flag leaf measurements were taken in Bozeman dryland and irrigated in 2009.

The number of fertile tillers was counted for a 0.3 m section of each plot after heading. Tiller counts and flag leaf measurements were taken in 2009 in Bozeman, MT only.

Biomass, Harvest Index, Test Weight, and Grain Yield

Biomass, harvest index, test weight, and grain yield were measured in Bozeman dryland, Bozeman irrigated, and Kalispell experiments in 2009. Biomass was measured

in the field directly before threshing. Plots were cut approximately at ground level and bundled. Bundles were weighed on a Toledo no springs, honest weight scale (Toledo Scales, Toledo, OH) directly before threshing. Plot grain weight was measured after hand-cleaning the seed from each plot.

Harvest index is a measure of grain compared to biomass and gives insight into the plants' ability to partition resources to grain development. Harvest index was calculated using the following equation:

$$Harvest\ Index = \frac{Plot\ grain\ weight}{Biomass}$$

Test weight is a volumetric measurement that is used to standardize grain for varying densities due to differences in weather or production. Hard red spring wheat has a standard test weight of 722.2 kg/m^3 (60lb/bu).

Test weight was measured using a Fairbanks grain weighing scale (Fairbanks Scales, Kansas City, MO). Test weights were corrected for varying grain densities using the following equations:

$$Test\ weight\ \left(\frac{lb}{bu}\right) = Test\ weight\ (g) * 0.4939$$

$$Test\ weight\ \left(\frac{kg}{m^3}\right) = Test\ weight\ \left(\frac{lb}{bu}\right) * 12.87$$

Yield was then calculated using the following equation:

$$Yield\ \left(\frac{kg}{ha}\right) = \left[\left(\frac{1.6}{Plot\ size}\right) * Plot\ grain\ weight\right] * 67.19$$

Biomass, harvest index, test weight, and grain yield measurements were made in 2009 for Bozeman and Kalispell, MT.

Grain Protein Percentage Analysis

Grain protein percentage analysis was performed on whole grains using a Foss Infratec 1241 Grain Analyzer (Tecator 1241, Foss Analytical AB, Höganäs, Sweden) in the Montana State University Cereal Quality Lab, Bozeman, MT. The Foss Infratec is a near infrared transmittance instrument that uses an indirect method to predict grain protein percentage based on electromagnetic radiation absorption of different grain constituents.

Statistical Analysis

Data was analyzed as a randomized block split plot design combined over environments using the Proc MIXED procedure in SAS Statistical Analysis Software, version 9.1.3 Service Pack 4, (© 2002-2003 by SAS Institute Inc., Cary, NC).

Mutant and wild type NILs were compared using CONTRAST and ESTIMATE statements in SAS and significance was determined at $\alpha = 0.05$. Interactions were analyzed via a Type 3 ANOVA.

CHAPTER 4

RESULTS

Data was analyzed via the Proc MIXED procedure utilizing CONTRAST and ESTIMATE statements in SAS Statistical Analysis Software to determine if there were significant differences both among and averaged across recurrent parents for each Rht genotype in an NIL population. Several questions were asked when analyzing data, among them: How do *Rh-B1b, Rht-D1b,* and *Rht8* compare to their respective wild types? How do *Rh-B1b, Rht-D1b,* and *Rht8* compare to each other. Interactions, such as environment, genetic background, and Rht genotype, were analyzed using a Type 3 ANOVA (Table 3, Appendix B).

Experimental Interactions

Environment was a significant variation in the means for every trait measured except coleoptile length and flag leaf length. For every trait, the recurrent parent was a significant source of variation in the means. Rht genotype was a significant source of variation in the means in all traits except flag leaf length and fertile tiller number.

There was a significant Environment*Recurrent parent interaction for plant height, yield, days to heading, test weight, and grain protein percentage. In addition, there was also a significant Environment*Rht genotype interaction for plant height, yield, days to heading, stem solidness, test weight, and grain protein percentage.

Table 3 Significance of Experimental Interactions (From Type 3 ANOVA)

	Env	Rparent	Rht	Env* Rparent	Env* Rht
Plant Height (cm)	<.0001	<.0001	<.0001	<.0001	<.0001
Yield (kg/ha)	<.0001	<.0001	<.0001	0.0478	<.0001
Coleoptile Length (cm)	.	0.0053	<.0001	.	.
Days to head	<.0001	<.0001	<.0001	0.0062	0.0042
Stem Solidness	0.0010	<.0001	<.0001	NS	0.0105
Flag Leaf Width (cm)	0.0076	<.0001	0.0242	NS	NS
Flag Leaf Length (cm)	NS	0.0013	NS	NS	NS
Harvest index	0.0500	0.0435	0.0143	NS	NS
Test weight (kg/m3)	<.0001	<.0001	0.0008	<.0001	0.0003
Fertile Tillers	0.0399	0.0109	NS	NS	NS
Grain protein	<.0001	<.0001	<.0001	<.0001	0.0055

	Rparent* Rht	Env* Rparent* Rht	REP(Env)	REP* Rparent (Env)
Plant Height (cm)	<.0001	0.0003	0.0007	0.0120
Yield (kg/ha)	<.0001	NS	0.0085	<.0001
Coleoptile Length (cm)	0.0265	.	0.0080	NS
Days to head	<.0001	<.0001	NS	<.0001
Stem Solidness	<.0001	NS	0.0009	<.0001
Flag Leaf Width (cm)	<.0001	NS	NS	NS
Flag Leaf Length (cm)	NS	NS	NS	NS
Harvest index	NS	NS	NS	NS
Test weight (kg/m3)	<.0001	0.0004	NS	0.0029
Fertile Tillers	NS	NS	NS	<.0001
Grain protein	<.0001	<.0001	NS	<.0001

Env= Environment; Rparent= Recurrent Parent; Rht= Rht Genotype

Plant Height

Each wild type was significantly different than its respective mutant. Wild types were not significantly different from each other when averaged across recurrent parents. *Rht-B1b* was significantly different than *Rht-D1b* in Amidon, Fortuna, and averaged across recurrent parents. *Rht-B1b* was significantly different than *Rht8* in all backgrounds. *Rht-D1b* was also significantly different than *Rht8* in all backgrounds. *Rht8* showed an average height of 82.3 cm and a height reduction of 10.1% as compared to *rht8*. *Rht-B1b* and *Rht-D1b* showed average heights of 73.6 cm and 71.8 cm and both had larger average height reductions than *Rht8* with reductions of 20.5% and 22.4%, respectively. Plant height means and contrast significance values are shown in Table 4.

Coleoptile Length

Rht-B1b and *Rht-D1b* significantly decreased coleoptile length by 21.3% and 22.8% and had an average length of 6.7 cm and 6.8 cm, respectively. On the other hand, *Rht8* had no significant effect on coleoptile length as compared to *rht8*. Coleoptiles were 9.1 cm in length, on average, for *Rht8*. *Rht-B1b* and *Rht-D1b* showed no significant difference from each other, while both were significantly different than *Rht8*. Coleoptile length means and contrast significance values are shown in Table 5.

Table 4 Plant Height (cm) Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	**Fortuna**	**JC73**	**Scholar**	**Mean**
Rht Genotype Means	*Rht-B1b* (mutant)	73.0	72.1	79.1	70.2	73.6
	Rht-B1a (wild type)	90.9	94.3	100.2	84.8	92.6
	Rht-D1b (mutant)	69.3	68.2	78.9	70.8	71.8
	Rht-D1a (wild type)	88.4	92.3	101.8	86.7	92.3
	Rht8 (mutant)	76.0	79.6	90.5	83.2	82.3
	rht8 (wild type)	87.9	91.2	102.4	85.1	91.6
	Recurrent parent	90.3	91.7	101.1	85.7	92.2
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	<.0001	<.0001	<.0001	<.0001	<.0001
	Rht-D1b vs. *Rht-D1a*	<.0001	<.0001	<.0001	<.0001	<.0001
	Rht8 vs. *rht8*	<.0001	<.0001	<.0001	0.0571	<.0001
	Rht-B1a vs. *Rht-D1a*	0.0122	0.0454	0.1156	0.0593	0.5953
	Rht-B1a vs. *rht8*	0.0026	0.0020	0.0304	0.8162	0.0615
	Rht-D1a vs. *rht8*	0.6068	0.2665	0.5501	0.0978	0.1794
	Rht-B1b vs. *Rht-D1b*	0.0003	0.0001	0.8551	0.5068	0.0005
	Rht-B1b vs. *Rht8*	0.0028	<.0001	<.0001	<.0001	<.0001
	Rht-D1b vs. *Rht8*	<.0001	<.0001	<.0001	<.0001	<.0001

Table 5 Coleoptile Length (cm) Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	**Fortuna**	**JC73**	**Scholar**	**Mean**
Rht Genotype Means	*Rht-B1b* (mutant)	7.2	6.7	6.1	6.9	6.7
	Rht-B1a (wild type)	7.6	9.6	8.7	8.2	8.6
	Rht-D1b (mutant)	7.1	6.8	6.7	6.7	6.8
	Rht-D1a (wild type)	9.3	9.4	8.5	8.1	8.8
	Rht8 (mutant)	9.7	9.2	8.4	8.9	9.1
	rht8 (wild type)	9.6	9.8	8.3	8.7	9.1
	Recurrent parent	8.5	9.9	8.6	8.5	8.9
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	0.4011	<.0001	<.0001	0.0105	<.0001
	Rht-D1b vs. *Rht-D1a*	<.0001	<.0001	0.0007	0.0074	<.0001
	Rht8 vs. *rht8*	0.8772	0.2778	0.7481	0.6173	0.9546
	Rht-B1a vs. *Rht-D1a*	0.0015	0.7115	0.6520	0.7760	0.2751
	Rht-B1a vs. *rht8*	0.0002	0.6844	0.3574	0.3921	0.0382
	Rht-D1a vs. *rht8*	0.5696	0.4383	0.6374	0.2553	0.3149
	Rht-B1b vs. *Rht-D1b*	0.8548	0.9190	0.2520	0.6815	0.7420
	Rht-B1b vs. *Rht8*	<.0001	<.0001	<.0001	0.0002	<.0001
	Rht-D1b vs. *Rht8*	<.0001	<.0001	0.0011	<.0001	<.0001

Stem Solidness

Stem solidness means and contrast significance values are found in Table 6. Averaged across recurrent parents, wild types were significantly different than their mutant counterpart and all wild types were also significantly different than each other. Contrast values showed little consistency among recurrent parents. Amidon and Fortuna had several contrasts showing significance differences while JC73 had a single significant difference. In all recurrent parents, *Rht-D1b* was significantly different than *Rht8. Rht-B1b* and *Rht-D1b* showed no significant difference but both were different than *Rht8*. Fortuna was the most solid with an average stem solidness of 19.3 for the recurrent parent and JC73 was the least solid with an average stem solidness of 8.2 for the recurrent parent. In every case but Scholar, *Rht8* lines had a lower average stem solidness score than the recurrent parent.

Days to Heading

Entries with Mara as a recurrent parent were screened for the photoperiod gene *Ppd-D1* as described in Materials and Methods. Those entries found to be photoperiod-insensitive were removed from the experiment. This ensured that differences in either Rht genotype or genetic background would account for any differences in days to heading and not differences in photoperiod response. Means and contrast significance values for days to heading are found in Table 7. *Rht-B1b* and *Rht-D1b* were significantly different

Table 6 Stem Solidness Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	**Fortuna**	**JC73**	**Scholar**	**Mean**
Rht Genotype Means	*Rht-B1b* (mutant)	18.3	18.4	8.7	13.3	14.7
	Rht-B1a (wild type)	17.6	11.0	8.2	12.1	12.2
	Rht-D1b (mutant)	18.1	15.8	8.9	14.2	14.3
	Rht-D1a (wild type)	15.0	11.7	8.1	11.5	11.6
	Rht8 (mutant)	12.9	19.5	7.7	12.2	13.1
	rht8 (wild type)	14.8	19.9	8.5	12.7	14.0
	Recurrent parent	14.2	19.3	8.2	13.5	13.8
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	0.2285	<.0001	0.4710	0.0458	<.0001
	Rht-D1b vs. *Rht-D1a*	<.0001	<.0001	0.1699	<.0001	<.0001
	Rht8 vs. *rht8*	0.0008	0.4692	0.1381	0.4240	0.0015
	Rht-B1a vs. *Rht-D1a*	<.0001	0.2164	0.8655	0.2640	0.0181
	Rht-B1a vs. *rht8*	<.0001	<.0001	0.6013	0.3185	<.0001
	Rht-D1a vs. *rht8*	0.7793	<.0001	0.4891	0.0351	<.0001
	Rht-B1b vs. *Rht-D1b*	0.6488	<.0001	0.6280	0.0964	0.1501
	Rht-B1b vs. *Rht8*	<.0001	0.0549	0.0930	0.0716	<.0001
	Rht-D1b vs. *Rht8*	<.0001	<.0001	0.0309	0.0006	<.0001

than their respective wild types averaged across backgrounds. On the other hand, there were no significant differences across backgrounds when comparing *Rht8* and *rht8*, except in Fortuna. *Rht8* didn't show consistent significance within differing backgrounds, indicating that *Rht8* has little to no effect on heading date.

Yield

Mean values and contrast significance values are shown in Table 8. *Rht-B1b* was significantly different than *Rht-B1a* in Fortuna, JC73, and averaged across recurrent parents. *Rht-D1b* was significantly different than Rht-D1a averaged across recurrent parents and in all backgrounds, except Scholar. *Rht8* only showed significant differences from its wild type in Amidon and averaged across recurrent parents. Averaged across recurrent parents, there were no significant differences between *Rht-B1a*, *Rht-D1a*, and *rht8*. *Rht-B1b* and *Rht-D1b* were significantly different in JC73 and averaged across recurrent parents. *Rht-B1b* and *Rht-D1b* were both significantly different than *Rht8* when averaged across recurrent parents and in all backgrounds but Scholar.

Rht-B1b and *Rht-D1b* caused average yield increases of 6.1% and 14.1%, respectively. *Rht8* caused a significant yield reduction of 21.0% and 5.3% in Amidon and averaged across recurrent parents, respectively.

Table 7 Days to Heading Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	Fortuna	JC73	Scholar	Mean
Rht Genotype Means	*Rht-B1b* (mutant)	58.1	56.9	60.3	58.9	58.6
	Rht-B1a (wild type)	57.6	56.6	60.2	58.5	58.2
	Rht-D1b (mutant)	57.6	57.9	62.5	59.6	59.4
	Rht-D1a (wild type)	57.2	56.9	62.0	58.8	58.7
	Rht8 (mutant)	57.7	55.9	61.8	58.2	58.4
	rht8 (wild type)	58.1	56.5	61.4	57.9	58.5
	Recurrent parent	57.5	56.8	60.1	58.5	58.2
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	0.0149	0.1956	0.6657	0.0848	0.0034
	Rht-D1b vs. *Rht-D1a*	0.0848	<.0001	0.0446	0.0011	<.0001
	Rht8 vs. *rht8*	0.0620	0.0219	0.0446	0.1506	0.7188
	Rht-B1a vs. *Rht-D1a*	0.1140	0.3138	<.0001	0.2498	<.0001
	Rht-B1a vs. *rht8*	0.0149	0.4716	<.0001	0.0043	0.0527
	Rht-D1a vs. *rht8*	<.0001	0.0848	0.0043	<.0001	0.0219
	Rht-B1b vs. *Rht-D1b*	0.0219	<.0001	<.0001	0.0066	<.0001
	Rht-B1b vs. *Rht8*	0.0620	<.0001	<.0001	0.0017	0.1720
	Rht-D1b vs. *Rht8*	0.6657	<.0001	0.0043	<.0001	<.0001

Table 8 Yield (kg/ha) Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	Fortuna	JC73	Scholar	Mean
Rht Genotype Means	*Rht-B1b* (mutant)	4839.1	4117.2	4834.0	4447.0	4559.3
	Rht-B1a (wild type)	4606.2	3609.5	4398.2	4576.4	4297.6
	Rht-D1b (mutant)	4899.2	4232.2	5464.1	4453.2	4762.2
	Rht-D1a (wild type)	4594.7	3372.5	4435.9	4289.4	4173.1
	Rht8 (mutant)	4031.0	3557.7	4176.4	4488.0	4063.3
	rht8 (wild type)	5104.0	3656.1	4160.7	4242.5	4290.8
	Recurrent parent	4770.4	3774.8	4368.8	4474.3	4347.1
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	0.0833	0.0002	0.0014	0.3341	0.0001
	Rht-D1b vs. *Rht-D1a*	0.0241	<.0001	<.0001	0.2219	<.0001
	Rht8 vs. *rht8*	<.0001	0.4625	0.9066	0.0681	0.0008
	Rht-B1a vs. *Rht-D1a*	0.9315	0.0781	0.7782	0.0333	0.0644
	Rht-B1a vs. *rht8*	0.0003	0.7277	0.0773	0.0135	0.9195
	Rht-D1a vs. *rht8*	0.0002	0.0354	0.0411	0.7260	0.0801
	Rht-B1b vs. *Rht-D1b*	0.6532	0.3905	<.0001	0.9625	0.0028
	Rht-B1b vs. *Rht8*	<.0001	<.0001	<.0001	0.7589	<.0001
	Rht-D1b vs. *Rht8*	<.0001	<.0001	<.0001	0.7949	<.0001

Flag Leaf Width

Flag leaf width showed little significant difference between or averaged across recurrent parents. *Rht8* and *rht8* were significantly different in JC73 and when averaged across recurrent parents. Neither *Rht-B1b* nor *Rht-D1b* showed any difference from their wild types between or averaged across recurrent parents. *Rht-B1a* and *rht8* showed significant difference in Fortuna and averaged across recurrent parents, and *Rht-B1b* and *Rht8* showed significant difference in Fortuna, JC73 and Scholar but not when averaged across recurrent parents. *Rht-D1b* and *Rht8* were only significantly different in the Amidon background. On average, Scholar had the widest flag leaves (ranging from 1.5 to 1.8 cm) and Fortuna had the narrowest (ranging from 1.2 to 1.5 cm). Flag leaf widths and contrast significance values are shown in Table 9.

Flag Leaf Length

Flag leaf length means and contrast significance values are shown in Table 10. There was little to no significance found either between or across recurrent parents, with the exception of the contrasts *Rht-D1b* vs. *Rht-D1a* and *Rht-B1b* vs. *Rht-D1b* in Scholar.

Harvest Index

Harvest index means are shown in Table 11. The general trend showed mutant types having a higher harvest index than their wild type counterparts, in all cases except *Rht8* and *rht8* in Amidon. Average increases of 10.6%, 18.2%, and 4.7% were shown for *Rht-B1b, Rht-D1b,* and *Rht8,* respectively.

Table 9 Flag Leaf Width (cm) Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	Fortuna	JC73	Scholar	Mean
Rht Genotype Means	*Rht-B1b* (mutant)	1.4	1.5	1.4	1.7	1.5
	Rht-B1a (wild type)	1.4	1.4	1.5	1.7	1.5
	Rht-D1b (mutant)	1.3	1.4	1.5	1.6	1.5
	Rht-D1a (wild type)	1.4	1.3	1.5	1.6	1.4
	Rht8 (mutant)	1.5	1.3	1.6	1.5	1.5
	rht8 (wild type)	1.4	1.2	1.4	1.6	1.4
	Recurrent parent	1.4	1.3	1.5	1.8	1.5
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	0.3724	0.0640	0.1458	0.7452	0.9352
	Rht-D1b vs. *Rht-D1a*	0.8709	0.0903	0.3724	0.1955	0.5698
	Rht8 vs. *rht8*	0.0903	0.0762	0.0033	0.2247	0.0095
	Rht-B1a vs. *Rht-D1a*	0.0903	0.5161	0.3724	0.6261	0.0640
	Rht-B1a vs. *rht8*	0.3307	0.0302	0.1249	0.2247	0.0037
	Rht-D1a vs. *rht8*	0.4652	0.1249	0.5161	0.4652	0.2741
	Rht-B1b vs. *Rht-D1b*	0.3307	0.4172	0.1458	0.0367	0.2247
	Rht-B1b vs. *Rht8*	0.1065	0.0247	0.0042	0.0067	0.8074
	Rht-D1b vs. *Rht8*	0.0106	0.1458	0.1458	0.5161	0.3307

Table 10 Flag Leaf Length (cm) Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	Fortuna	JC73	Scholar	Mean
Rht Genotype Means	*Rht-B1b* (mutant)	16.9	17.9	17.9	17.9	17.6
	Rht-B1a (wild type)	17.0	18.0	18.4	18.5	18.0
	Rht-D1b (mutant)	15.4	17.5	17.3	25.0	18.8
	Rht-D1a (wild type)	15.7	17.4	17.1	19.0	17.3
	Rht8 (mutant)	16.2	16.8	17.4	18.4	17.2
	rht8 (wild type)	16.6	16.5	17.0	19.1	17.3
	Recurrent parent	15.2	18.3	18.7	19.8	18.0
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	0.9478	0.9373	0.7832	0.7615	0.7178
	Rht-D1b vs. *Rht-D1a*	0.9096	0.9582	0.9165	0.0023	0.1161
	Rht8 vs. *rht8*	0.8373	0.8561	0.8476	0.7334	0.9312
	Rht-B1a vs. *Rht-D1a*	0.4716	0.7631	0.5058	0.7782	0.4824
	Rht-B1a vs. *rht8*	0.8322	0.4198	0.4649	0.7731	0.4649
	Rht-D1a vs. *rht8*	0.6110	0.6126	0.9478	0.9948	0.9773
	Rht-B1b vs. *Rht-D1b*	0.4427	0.8647	0.7748	0.0003	0.2170
	Rht-B1b vs. *Rht8*	0.7252	0.5838	0.7916	0.8017	0.6482
	Rht-D1b vs. *Rht8*	0.6767	0.7056	0.9826	0.0008	0.0923

When averaged across recurrent parents, each mutant was significantly different than its wild type. Among recurrent parents, each mutant was different than its wild type except in Amidon *Rht-B1b* and *Rht-B1a* and in all of Scholar. There were significant differences both among and averaged across recurrent parents when comparing wild types. *Rht-B1a* and *Rht-D1a* were significantly different in Fortuna, Scholar, and averaged across recurrent parents. *Rht-B1a* and *rht8* were significantly different only in Amidon, and *Rht-D1a* and *rht8* were significantly different in all but JC73. When comparing mutants, significant differences were found both among and averaged across recurrent parents. *Rht-B1b* and *Rht-D1b* were significantly different in Amidon and Fortuna. *Rht-B1b* and *Rht8* were significantly different in Fortuna, JC73, and averaged across recurrent parents. *Rht-D1b* and *Rht8* were significantly different in Amidon, JC73, and averaged across recurrent parents.

Fertile Tillers

There were no significant differences between mutant and wild type when averaged across recurrent parents. Results were consistent between recurrent parents with the exception of Amidon and Fortuna. Amidon showed a significance differences between *Rht-B1b* and *Rht-B1a* and *Rht-B1b* and *Rht8*. Fortuna showed significance in *Rht-D1b* and *Rht-D1a*. The only significant difference when averaged across recurrent parents was in *Rht-D1b* and *Rht8*, with *Rht8* causing a reduction in the number of fertile tillers. Data are shown in Table 12. As with flag leaf length, there was little significant

Table 11 Harvest Index Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	Fortuna	JC73	Scholar	Mean
Rht Genotype Means	*Rht-B1b* (mutant)	0.39	0.45	0.42	0.38	0.41
	Rht-B1a (wild type)	0.37	0.39	0.36	0.37	0.37
	Rht-D1b (mutant)	0.42	0.42	0.44	0.36	0.41
	Rht-D1a (wild type)	0.38	0.33	0.34	0.34	0.35
	Rht8 (mutant)	0.58	0.41	0.40	0.38	0.44
	rht8 (wild type)	0.41	0.39	0.34	0.36	0.37
	Recurrent parent	0.40	0.40	0.34	0.38	0.38
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	0.0626	<.0001	<.0001	0.3661	<.0001
	Rht-D1b vs. *Rht-D1a*	0.0005	<.0001	<.0001	0.0478	<.0001
	Rht8 vs. *rht8*	0.0155	0.0255	<.0001	0.0957	0.0017
	Rht-B1a vs. *Rht-D1a*	0.1867	<.0001	0.1252	0.0064	<.0001
	Rht-B1a vs. *rht8*	0.0001	0.8148	0.0939	0.4934	0.5140
	Rht-D1a vs. *rht8*	0.0106	<.0001	0.8858	0.0391	<.0001
	Rht-B1b vs. *Rht-D1b*	0.0029	0.0131	0.1036	0.0952	0.8121
	Rht-B1b vs. *Rht8*	0.6826	0.0002	0.0138	0.9335	0.0012
	Rht-D1b vs. *Rht8*	0.0008	0.1960	<.0001	0.0800	0.0005

difference in the number of fertile tillers either between or averaged across recurrent parents.

Test Weight

Test weights were well within the standard range for hard red spring wheat (standard test weight is 772.2kg/m^3) with the average of each Rht genotype being between 772.4 to 778.3 kg/m^3. No significant differences were seen in wild types compared to their mutant counterparts when averaged across genotypes, but all were significant in the Amidon background. Rht genotype didn't have a consistent effect between recurrent parents, as shown in Table 13. *Rht-B1b* was significantly different than *Rht-D1b* in JC73 and *Rht8* in JC73 and Scholar and averaged across recurrent parents. Additionally, significant differences were seen among *Rht-B1a* vs. *Rht-D1a* and *Rht-B1a* vs. *rht8* in Fortuna and averaged across recurrent parents.

Grain Protein Percentage

Grain protein percentage means and contrast values are found in Table 14. Data showed significant differences between each mutant type and its wild type. In each case, except for Amidon *Rht8*, the Rht mutant type caused a significant decrease in grain protein compared to the wild type both among and averaged across recurrent parents. The average decreases in grain protein were 7.8%, 11.8%, and 3.1% for *Rht-B1*, *Rht-D1*, and *Rht8*, respectively. In addition, *Rht-B1b*, *Rht-D1b*, and *Rht8* were all significantly different than each other.

Table 12 Fertile Tillers (Heads/Ft) Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	**Fortuna**	**JC73**	**Scholar**	**Mean**
Rht Genotype Means	*Rht-B1b* (mutant)	62.0	56.7	48.3	50.6	54.4
	Rht-B1a (wild type)	52.4	58.3	48.4	51.9	52.8
	Rht-D1b (mutant)	60.8	62.2	46.6	51.5	55.3
	Rht-D1a (wild type)	57.4	54.1	44.0	56.8	53.1
	Rht8 (mutant)	54.0	58.4	41.5	50.3	51.1
	rht8 (wild type)	57.7	54.7	42.8	51.1	51.6
	Recurrent parent	58.3	54.5	47.7	51.5	53.0
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	0.0146	0.6819	0.9828	0.7300	0.3949
	Rht-D1b vs. *Rht-D1a*	0.3889	0.0385	0.5040	0.1693	0.2633
	Rht8 vs. *rht8*	0.3435	0.3327	0.7300	0.8460	0.7957
	Rht-B1a vs. *Rht-D1a*	0.1973	0.2820	0.2543	0.2048	0.8629
	Rht-B1a vs. *rht8*	0.1760	0.3545	0.1504	0.8292	0.5389
	Rht-D1a vs. *rht8*	0.9484	0.8799	0.7626	0.1387	0.4316
	Rht-B1b vs. *Rht-D1b*	0.7462	0.1565	0.6506	0.8124	0.6584
	Rht-B1b vs. *Rht8*	0.0405	0.6506	0.0792	0.9484	0.0867
	Rht-D1b vs. *Rht8*	0.0829	0.3327	0.1900	0.7626	0.0321

Table 13 Test Weight (kg/m^3) Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	Fortuna	JC73	Scholar	Mean
Rht Genotype Means	*Rht-B1b* (mutant)	767.7	782.6	773.7	783.2	776.8
	Rht-B1a (wild type)	777.5	788.7	760.8	786.1	778.3
	Rht-D1b (mutant)	768.6	776.6	759.4	784.9	772.4
	Rht-D1a (wild type)	776.9	778.5	753.5	788.2	774.3
	Rht8 (mutant)	768.3	783.9	750.0	792.8	773.7
	rht8 (wild type)	775.8	782.0	754.4	788.3	775.1
	Recurrent parent	782.5	792.6	744.7	791.8	777.9
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	0.0018	0.0533	<.0001	0.3459	0.3431
	Rht-D1b vs. *Rht-D1a*	0.0083	0.5394	0.0591	0.286	0.2206
	Rht8 vs. *rht8*	0.0165	0.5469	0.1566	0.1533	0.3666
	Rht-B1a vs. *Rht-D1a*	0.8556	0.0013	0.0203	0.5026	0.0114
	Rht-B1a vs. *rht8*	0.5932	0.0330	0.0399	0.4811	0.0444
	Rht-D1a vs. *rht8*	0.7245	0.2661	0.7849	0.9728	0.5932
	Rht-B1b vs. *Rht-D1b*	0.7502	0.0547	<.0001	0.5853	0.0051
	Rht-B1b vs. *Rht8*	0.8289	0.6907	<.0001	0.0024	0.0493
	Rht-D1b vs. *Rht8*	0.9185	0.0209	0.0027	0.0121	0.3911

Table 14 Grain Protein (%) Means and Contrast Significance Values Within and Across Recurrent Parents

		Amidon	**Fortuna**	**JC73**	**Scholar**	**Mean**
Rht Genotype Means	*Rht-B1b* (mutant)	15.0	14.2	13.2	15.5	14.5
	Rht-B1a (wild type)	15.6	15.9	14.9	16.3	15.7
	Rht-D1b (mutant)	13.8	14.4	12.8	15.6	14.2
	Rht-D1a (wild type)	15.6	16.3	15.4	16.9	16.1
	Rht8 (mutant)	15.8	15.4	14.3	16.0	15.4
	rht8 (wild type)	15.5	15.9	15.2	16.3	15.7
	Recurrent parent	15.8	16.0	15.1	16.2	15.8
Contrast Significance Values	*Rht-B1b* vs. *Rht-B1a*	<.0001	<.0001	<.0001	<.0001	<.0001
	Rht-D1b vs. *Rht-D1a*	<.0001	<.0001	<.0001	<.0001	<.0001
	Rht8 vs. *rht8*	0.0002	<.0001	<.0001	0.001	<.0001
	Rht-B1a vs. *Rht-D1a*	1.0000	0.0006	<.0001	<.0001	<.0001
	Rht-B1a vs. *rht8*	0.0750	0.9050	0.0056	0.8113	0.4929
	Rht-D1a vs. *rht8*	0.0750	0.0008	0.0381	<.0001	<.0001
	Rht-B1b vs. *Rht-D1b*	<.0001	0.0577	<.0001	0.0577	<.0001
	Rht-B1b vs. *Rht8*	<.0001	<.0001	<.0001	<.0001	<.0001
	Rht-D1b vs. *Rht8*	<.0001	<.0001	<.0001	0.0003	<.0001

CHAPTER 5

DISCUSSION

As expected, Rht genotype has a significant effect on plant height. Also, height reductions were similar to those reported in the literature. Worland and Law (1986) and Worland et al (1998) reported a height reduction of approximately 10% due to *Rht8*. This study found a height reduction of 10.1% for *Rht8*. *Rht-B1b* and *Rht-D1b* both had larger average height reductions of 20.5% and 22.4%, respectively, which are in the mid range of values reported in the literature (Blake et al., 2009; Flintham et al., 1996; Gale and Youseffian, 1985; Trethowan et al., 2001).

Crop establishment is a major determinant of yield and coleoptile length is an important factor in seedling emergence, making coleoptile length a trait of importance to plant breeders. Rht genotype is a major factor in final coleoptile length. In agreement with the literature, it was found that *Rht8* has no significant effect on coleoptile length as compared to *rht8*. On the other hand, *Rht-B1b* and *Rht-D1b* both cause a reduction in length of 21.3% and 22.8%, respectively. Because *Rht8* showed no reduction in final coleoptile length, it could prove useful in environments where height reduction is still needed and deeper sowing is advantageous. In dry environments, farmers sowing seed into a receding moisture profile could utilize *Rht8* to allow for planting below the drying topsoil, insuring contact with available soil moisture.

Rht genotype also had a highly significant effect on yield. *Rht-B1b* and *Rht-D1b* both had a positive effect on yield, increasing yield an average of 6.1% and 14.1%, respectively compared to wild type. Surprisingly, in contrast to other published studies,

Rht8 had a negative effect on yield in half of the recurrent parents and also averaged over all recurrent parents. The average reduction in yield was 5.3%. This data suggests that *Rht8* is not always advantageous in terms of yield. However, there are several possible explanations for this apparent yield penalty associated with *Rht8*.

First, the data may be skewed towards a negative yield potential due to the Amidon *Rht8* and *rht8* lines. There was a -20.0% difference between the yields of Amidon *Rht8* and *rht8* lines, while there are much smaller differences in the other recurrent parents. Yield differences of -2.7%, +0.4%, and +5.8% were seen in Fortuna, JC73, and Scholar, respectively. Removing the Amidon data gives an average + 3.5% change in yield due to *rht8*.

Another possible explanation for the negative yield potential of *Rht8* could be due to environmental conditions. Recall that environmental effects were a significant experimental interaction (Table 3). Increased yield potential for *Rht-B1b* and *Rh-D1b* has been noted under high-input growing conditions (Knott, 1986; Hedden, 2003; McNeal et al., 1972) In areas with heat or drought stress, standard height varieties perform better than those containing *Rht-B1b* or *Rht-D1b* (McNeal et al., 1972). Because of its slight reduction in height, *Rht8* may perform in a similar way as standard height wheats- i.e. it may have greater yield stability in low-input or high heat/drought environments. Much of the studies investigating yield potential of *Rht8* have been carried out in Australia, which experiences both high heat and drought during the growing season. The growing 2009 growing season was much cooler and wetter than average for Bozeman, MT which could account for the unexpected yield results seen in the *Rht8* lines. Furthermore, Bozeman

and Kalispell do not represent the typical environmental conditions found in the grain growing regions of Montana and the Northern Great Plains.

A third explanation for the yield penalty due to *Rht8* could be due to the fact that it is functionally different than both *Rht-B1b* and *Rht-D1b*. *Rht-B1b* and *Rht-D1b* are caused by nonsense mutations in the N-terminal region of their encoded GA-signaling DELLA proteins, and both lead to GA-insensitivity. *Rht8*, on the other hand, acts through a different, as yet unknown, mechanism and is GA-sensitive. Gibberellic acid controls many aspects of plant growth and development, including many factors related to yield, such as plant height and coleoptile length. Elucidating the functional mechanism of *Rht8* could give clues as to how it influences yield. These fundamental differences between *Rht-B1b*, *Rht-D1*, and *Rht8,* the environmental factors previously discussed, and the Amidon *Rht8*/*rht8* data could give insight into the conflicting results in yield potential.

Rht genotype showed little to no effect on several of the agronomic characteristics considered in this study, including number of days until heading, stem solidness, flag leaf width and length, number of fertile tillers, and test weight. In each case, there was little consistency in statistical significance of the contrasts among recurrent parents. A possible explanation arises from the nature of near-isogenic lines. The population in this study contained BC4 and BC5 lines (Appendix D), containing 3.1% and 1.6% donor genes, respectively. This genetic variation among near-isogenic lines could account for some of the variation seen in lines with the same Rht genotype among the different recurrent parents.

Grain protein results followed the typical negative correlation between yield and grain protein (Terman et al, 1969). Higher yielding varieties tend to have a lower grain protein percentage than lower yielding varieties. Taking into consideration the protein-yield correlation and this study's yield data, one would assume that the *Rht-B1b* and *Rht-D1b* lines would have a lower percentage of grain protein and that the *Rht8* lines would have a higher percentage than compared to their wild types. *Rht-B1b* and *Rht-D1b* did a significantly lower grain protein percentage than *Rht-B1a* and *Rht-D1a*. However, *Rht8* also had lower grain protein percentage than *rht8* when averaged across recurrent parents. Among recurrent parents, only Fortuna showed results contrary to the expected relationship between yield and grain protein percentage. The remaining recurrent parents showed either an increase or a decrease in yield and the inverse result in grain protein percentage, as expected.

Table 15 summarizes changes due to the Rht mutant genotype for plant height, coleoptile length, stem solidness, yield and grain protein within each recurrent parent and averaged across recurrent parents. Using such a table, a plant breeder can weigh the advantage of each Rht genotype over a series of traits, allowing one to find the optimal Rht genotype for a particular background. For instance, in the case of Scholar, *Rht8* would be the optimal Rht genotype because of its positive yield potential, negligible effect on coleoptile length, and small reduction in height.

Table 15 Percent Change as Compared to Wild Type in Traits influenced by Rht Genotype

Recurrent Parent	Trait	*Rht-B1b*	*Rht-D1b*	*Rht8*
Amidon	Coleoptile Length	-5.7	-23. 8**	+0.8
	Plant Height	-19.7**	-21.6**	-13. 5**
	Stem Solidness	+4.0	+20.1**	-12.8**
	Yield	+5.1**	+6.6**	-21.0**
	Grain Protein	-4.4**	-11.5**	+2.3**
Fortuna	Coleoptile Length	-28.9**	-27.9**	-5.7
	Plant Height	-23.5**	-26.1**	-12.7**
	Stem Solidness	+66.3**	35.0**	-2.0
	Yield	+14.1**	+25.5**	-2.7
	Grain Protein	-10.7**	-11.4**	-3.2**
JC73	Coleoptile Length	-30.1**	-21.3**	+1.9
	Plant Height	-21.1**	-22.5**	-11.6**
	Stem Solidness	+6.1	+9.9	-9.4
	Yield	+9.9**	+23.2**	+0.4
	Grain Protein	-11.3**	-17.0**	-5.8**
Scholar	Coleoptile Length	-16.3*	-17.4**	+3.0
	Plant Height	-17.3**	-18.3**	-2.3
	Stem Solidness	+10.0**	+23.5**	-3.9
	Yield	-2.8	+3.8	+5.8
	Grain Protein	-5.0**	-7.8**	-1.9**
Mean	Coleoptile Length	-21.3**	-22.8**	-0.2
	Plant Height	-20.5**	-22.4**	-10.1**
	Stem Solidness	+19.7**	+23.2**	-6.5**
	Yield	+6.1**	+14.1**	-5.3**
	Grain Protein	-7.8**	-11.8**	-3.1**

* significant at 0.05, ** significant at 0.01

Conclusion

Arguably the most economically important agronomic traits in spring wheat breeding are those that effect yield. Yield is a complex and multi-genic trait that is affected by many other plant characteristics and environmental factors. Because of the quantitative nature of yield, it does not depend only on the presence of dwarfing genes by themselves, but also on genetic background and environmental variables.

This study suggests that *Rht-B1b* and *Rht-D1b* are superior, in terms of yield potential, to *Rht8.* However, additional evaluation of *Rht8* in differing environments is needed to elucidate its precise effects on yield, yield components, and other agronomic traits.

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APPENDICES

APPENDIX A

MEANS BY LOCATION AVERAGED ACROSS RECURRENT PARENTS

Bozeman, MT 2008

Rht genotype	Days to Heading	Plant Height (cm)	Yield (kg/ha)	Stem Sol.	FL Width (cm)	FL Length (cm)	Heads/ft	Harvest Index	Test weight	Protein %
Dryland										
Rht-B1b (mutant)	65.7	74.4	-	14.2	-	-	-	-	-	-
Rht-B1a (wild type)	65.0	96.6	-	11.2	-	-	-	-	-	-
Rht-D1b (mutant)	66.5	71.4	-	14.1	-	-	-	-	-	-
Rht-D1a (wild type)	65.7	96.1	-	10.7	-	-	-	-	-	-
Rht8 (mutant)	65.3	87.5	-	12.4	-	-	-	-	-	-
rht8 (wild type)	65.3	96.4	-	11.7	-	-	-	-	-	-
Recurrent parent	65.3	95.8	-	12.4	-	-	-	-	-	-
Irrigated										
Rht-B1b (mutant)	59.9	77.0	-	14.0	-	-	-	-	-	-
Rht-B1a (wild type)	59.5	96.0	-	11.9	-	-	-	-	-	-
Rht-D1b (mutant)	60.8	73.9	-	14.8	-	-	-	-	-	-
Rht-D1a (wild type)	60.0	94.1	-	11.9	-	-	-	-	-	-
Rht8 (mutant)	59.5	83.3	-	13.8	-	-	-	-	-	-
rht8 (wild type)	59.6	93.5	-	14.4	-	-	-	-	-	-
Recurrent parent	59.2	94.3	-	13.3	-	-	-	-	-	-

Bozeman, MT 2009

Rht genotype	Days to Heading	Plant Height (cm)	Yield (kg/ha)	Stem Sol.	FL Width (cm)	FL Length (cm)	Heads/ft	Harvest Index	Test weight	Protein %
Dryland										
Rht-B1b (mutant)	58.9	75.5	4988.3	17.5	1.5	17.3	52.2	43.0	788.9	13.5
Rht-B1a (wild type)	59.0	92.5	4424.9	14.9	1.5	17.2	49.1	38.9	795.7	14.9
Rht-D1b (mutant)	60.0	74.6	5307.5	16.5	1.4	16.5	52.8	43.6	784.8	13.2
Rht-D1a (wild type)	59.3	94.4	4472.5	14.1	1.4	16.7	49.9	37.9	792.5	15.2
Rht8 (mutant)	59.5	84.3	4361.7	15.5	1.4	16.7	47.5	40.7	789.6	14.4
rht8 (wild type)	59.0	93.3	4562.1	17.2	1.3	16.7	49.2	39.0	791.0	14.8
Recurrent parent	58.8	92.7	4485.5	15.7	1.4	18.0	52.8	38.6	797.8	15.0
Irrigated										
Rht-B1b (mutant)	59.5	74.6	5631.2	12.0	1.5	18.0	56.6	41.8	790.2	13.9
Rht-B1a (wild type)	58.5	92.0	5115.1	9.8	1.5	18.8	56.4	37.0	789.6	15.1
Rht-D1b (mutant)	59.5	75.1	6010.5	11.0	1.5	21.1	57.7	41.9	786.4	13.6
Rht-D1a (wild type)	59.3	93.6	5202.2	9.0	1.5	17.9	56.3	36.5	790.1	15.4
Rht8 (mutant)	58.8	83.5	4898.0	10.2	1.5	17.8	54.7	54.0	787.0	14.8
rht8 (wild type)	59.3	91.8	5221.5	11.9	1.5	17.8	53.9	37.5	789.2	15.2
Recurrent parent	58.6	92.6	5203.9	11.6	1.5	18.0	53.3	38.7	785.3	15.2

Kalispell, MT 2009

Rht genotype	Days to Heading	Plant Height (cm)	Yield (kg/ha)	Stem Sol.	FL Width (cm)	FL Length (cm)	Heads/ft	Harvest Index	Test weight	Protein %
Rht-B1b (mutant)	48.9	66.5	3058.5	15.6	-	-	-	38.4	751.3	16.0
Rht-B1a (wild type)	49.2	85.7	3352.7	13.4	-	-	-	35.5	749.5	17.0
Rht-D1b (mutant)	50.2	64.0	2968.5	14.9	-	-	-	38.0	745.9	15.7
Rht-D1a (wild type)	49.4	83.2	2844.7	12.2	-	-	-	30.2	740.3	17.5
Rht8 (mutant)	49.0	73.0	2930.0	13.5	-	-	-	37.6	744.5	17.0
rht8 (wild type)	49.1	83.0	3088.8	14.7	-	-	-	36.0	745.2	17.2
Recurrent parent	49.3	85.6	3351.8	16.0	-	-	-	37.1	750.5	17.3

APPENDIX B

TYPE 3 ANALYSIS OF VARIANCE

	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Plant Height	Env	4	6803.666	1700.917	28.99	<.0001
	Rparent	3	10015.000	3338.404	253.14	<.0001
	Env*Rparent	12	3731.167	310.931	23.58	<.0001
	Rht Genotype	6	31006.000	5167.641	685.36	<.0001
	Rparent*Rht Genotype	18	1783.931	99.107	13.14	<.0001
	Env*Rht Genotype	24	641.246	26.719	3.54	<.0001
	Envt*Rparent*Rht Genotype	72	1010.920	14.041	1.86	0.0003
	REP(Env)	10	586.753	58.675	4.45	0.0007
	REP*Rparent(Env)	30	395.640	13.188	1.75	0.0120
Yield	Env	2	222377381.000	111188690.000	93.17	<.0001
	Rparent	3	32118518.000	10706173.000	37.36	<.0001
	Env*Rparent	6	4636980.000	772830.000	2.70	0.0478
	Rht Genotype	6	11994328.000	1999055.000	24.91	<.0001
	Rparent*Rht Genotype	18	11657376.000	647632.000	8.07	<.0001
	Env*Rht Genotype	12	9794672.000	816223.000	10.17	<.0001
	Envt*Rparent*Rht Genotype	36	3297330.000	91593.000	1.14	0.2878
	REP(Env)	6	7160177.000	1193363.000	4.16	0.0085
	REP*Rparent(Env)	18	5158729.000	286596.000	3.57	<.0001
Coleoptile Length	Env	0	0.000	0.000	0.00	.
	Rparent	3	13.566	4.522	8.56	0.0053
	Env*Rparent	0	0.000	0.000	0.00	.
	Rht Genotype	6	104.883	17.481	33.53	<.0001
	Rparent*Rht Genotype	18	18.094	1.005	1.93	0.0265
	Env*Rht Genotype	0	0.000	0.000	0.00	.
	Envt*Rparent*Rht Genotype	0	0.000	0.000	0.00	.
	REP(Env)	3	11.919	3.973	7.52	0.0080
	REP*Rparent(Env)	9	4.754	0.528	1.01	0.4378
Days to Heading	Env	4	11503.000	2875.861	1324.05	<.0001
	Rparent	3	1136.773	378.924	102.62	<.0001
	Env*Rparent	12	136.313	11.359	3.08	0.0062
	Rht Genotype	6	59.217	9.869	24.63	<.0001
	Rparent*Rht Genotype	18	97.764	5.431	13.55	<.0001
	Env*Rht Genotype	24	19.456	0.811	2.02	0.0042
	Envt*Rparent*Rht Genotype	72	59.254	0.823	2.05	<.0001
	REP(Env)	10	21.720	2.172	0.59	0.8106
	REP*Rparent(Env)	30	110.780	3.693	9.22	<.0001
Stem Solidness	Env	4	1276.225	319.056	11.23	0.0010
	Rparent	3	4351.857	1450.619	220.33	<.0001
	Env*Rparent	12	163.261	13.605	2.07	0.0529
	Rht Genotype	6	456.029	76.005	31.17	<.0001
	Rparent*Rht Genotype	18	1348.835	74.935	30.73	<.0001
	Env*Rht Genotype	24	108.913	4.538	1.86	0.0105
	Envt*Rparent*Rht Genotype	72	206.007	2.861	1.17	0.1878
	REP(Env)	10	284.010	28.401	4.31	0.0009
	REP*Rparent(Env)	30	197.514	6.584	2.70	<.0001

	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Flag Leaf Width	Env	1	0.269	0.269	24.73	0.0076
	Rparent	3	1.951	0.650	72.11	<.0001
	Env*Rparent	3	0.003	0.001	0.11	0.9517
	Rht Genotype	6	0.120	0.020	2.56	0.0242
	Rparent*Rht Genotype	18	0.474	0.026	3.35	<.0001
	Env*Rht Genotype	6	0.058	0.010	1.23	0.2966
	Envt*Rparent*Rht Genotype	18	0.097	0.005	0.69	0.8180
	REP(Env)	4	0.044	0.011	1.21	0.3579
	REP*Rparent(Env)	12	0.108	0.009	1.15	0.3306
Flag Leaf Length	Env	1	90.127	90.127	4.60	0.0985
	Rparent	3	264.811	88.270	10.21	0.0013
	Env*Rparent	3	55.975	18.658	2.16	0.1461
	Rht Genotype	6	47.466	7.911	0.73	0.6278
	Rparent*Rht Genotype	18	213.176	11.843	1.09	0.3734
	Env*Rht Genotype	6	78.493	13.082	1.20	0.3106
	Envt*Rparent*Rht Genotype	18	222.278	12.349	1.14	0.3300
	REP(Env)	4	78.365	19.591	2.27	0.1225
	REP*Rparent(Env)	12	103.771	8.648	0.80	0.6532
Harvest Index	Env	2	1168.104	584.052	5.14	0.0500
	Rparent	3	1236.808	412.269	3.31	0.0435
	Env*Rparent	6	844.176	140.696	1.13	0.3841
	Rht Genotype	6	2101.084	350.181	2.76	0.0143
	Rparent*Rht Genotype	18	2465.662	136.981	1.08	0.3777
	Env*Rht Genotype	12	1525.718	127.143	1.00	0.4496
	Envt*Rparent*Rht Genotype	36	4736.800	131.578	1.04	0.4234
	REP(Env)	6	681.357	113.560	0.91	0.5080
	REP*Rparent(Env)	18	2239.289	124.405	0.98	0.4847
Test Weight	Env	2	631.252	315.626	246.12	<.0001
	Rparent	3	219.311	73.104	119.46	<.0001
	Env*Rparent	6	109.098	18.183	29.71	<.0001
	Rht Genotype	6	6.407	1.068	4.09	0.0008
	Rparent*Rht Genotype	18	46.543	2.586	9.91	<.0001
	Env*Rht Genotype	12	10.262	0.855	3.28	0.0003
	Envt*Rparent*Rht Genotype	36	21.257	0.590	2.26	0.0004
	REP(Env)	6	7.694	1.282	2.10	0.1046
	REP*Rparent(Env)	18	11.015	0.612	2.34	0.0029

	Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Fertile Tillers	Env	1	1077.680	1077.680	9.00	0.0399
	Rparent	3	3848.171	1282.724	5.80	0.0109
	Env*Rparent	3	99.266	33.089	0.15	0.9279
	Rht Genotype	6	309.577	51.596	1.16	0.3345
	Rparent*Rht Genotype	18	874.506	48.584	1.09	0.3723
	Env*Rht Genotype	6	196.226	32.704	0.73	0.6227
	Envt*Rparent*Rht Genotype	18	1130.952	62.831	1.41	0.1436
	REP(Env)	4	478.988	119.747	0.54	0.7084
	REP*Rparent(Env)	12	2652.964	221.080	4.97	<.0001
Grain Protein	Env	2	285.368	142.684	923.62	<.0001
	Rparent	3	92.409	30.803	150.36	<.0001
	Env*Rparent	6	13.161	2.194	10.71	<.0001
	Rht Genotype	6	113.624	18.937	487.36	<.0001
	Rparent*Rht Genotype	18	21.461	1.192	30.68	<.0001
	Env*Rht Genotype	12	1.158	0.096	2.48	0.0055
	Envt*Rparent*Rht Genotype	36	4.590	0.127	3.28	<.0001
	REP(Env)	6	0.927	0.154	0.75	0.6145
	REP*Rparent(Env)	18	3.688	0.205	5.27	<.0001

APPENDIX C

BACKCROSS STATUS OF NEAR-ISOGENIC LINES

Recurrent Parent	Donor Parent	Rht Genotype	Backcross generation	% Donor Genes	% Recurrent Parent Genes
Amidon	HiLine	*Rht-B1b*	BC4	3.13	96.87
	McNeal	*Rht-D1b*			
	Mara	*Rht8*	BC5	1.56	98.44
Fortuna	HiLine	*Rht-B1b*	BC4	3.13	96.87
	McNeal	*Rht-D1b*			
	Mara	*Rht8*	BC5	1.56	98.44
JC73	HiLine	*Rht-B1b*			
	McNeal	*Rht-D1b*	BC5	1.56	98.44
	Mara	*Rht8*			
Scholar	HiLine	*Rht-B1b*			
	McNeal	*Rht-D1b*	BC5	1.56	98.44
	Mara	*Rht8*			
Thatcher	HiLine	*Rht-B1b*			
	McNeal	*Rht-D1b*	BC5	1.56	98.44
	Mara	*Rht8*			