DISSERTATION

TRAIT ASSOCIATIONS AND ALLELIC DIVERSITY AT THE XGWM 261, *RHT-B1*, AND *RHT-D1* LOCI IN WINTER WHEAT

Submitted by

Sally R. Clayshulte

Department of Soil and Crop Sciences

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SALLY R. CLAYSHULTE ENTITLED

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Committee on Graduate Work

Mark A. Brick

Mark Brick, Ph.D.

Jerry J. Johnson

Jerry Johnson, Ph.D.

Cecil Stushnoff, Ph.D.

Scott Haley

Adviser, Scott Haley, Ph.D.

G. A. Peterson

Department Head, Gary Peterson, PhD.

ABSTRACT OF DISSERTATION

TRAIT ASSOCIATIONS AND ALLELIC DIVERSITY AT THE XGWM 261, *RHT-B1*, AND *RHT-D1* LOCI IN WINTER WHEAT

The semi-dwarfing genes *Rht-B1b* and *Rht-Dlb* have been used extensively in the development of reduced height wheat (*Triticum aestivum* L.) cultivars. Yield increases have been observed through decreased lodging and improved partitioning of assimilates, although reduced stand from shorter coleoptiles and yield instability when grown in low yielding environments have been cited as disadvantages. Interest in the gibberellic acid sensitive semi-dwarfing gene *Rht8* has increased since the discovery of Xgwm261, a closely linked microsatellite marker, which may be useful as a diagnostic marker for *Rht8*. Two studies were conducted to assess allelic variation and trait associations of the Xgwm 261, *Rht-B1* and *Rht-D1,* loci in winter wheat.

The objectives of the first study were to determine allelic variation at the Xgwm 261 marker locus among historical and current Great Plains hard winter wheat germplasm, to characterize alleles at the *Rht-B1* and *Rht-D1* loci, and to evaluate the effects of these semi-dwarfing alleles on plant height, gibberellic acid (GA) sensitivity, and coleoptile length. Microsatellite markers Xgwm 261 (*Rht8*), BF-MR1 (*Rht-B1*), and DF-MR2 (*Rht-D1*) were used to determine the allelic identity at the *Rht8*, *Rht-B1* and *Rht-D1* loci. Plant height, GA sensitivity, and coleoptile length were evaluated in greenhouse and growth room conditions. Four allelic classes were characterized with the Xgwm 261 marker with fragment sizes of 165 bp, 174 bp, 192 bp, and 210 bp among 72 Great Plains entries: alleles at the *Rht-B1* and *Rht-D1* loci were also determined. This study was unable to verify the diagnostic capability of the Xgwm 261-192 bp microsatellite marker to identify germplasm with reduced height, gibberellic acid sensitivity, and a longer coleoptile suggesting the lack of linkage between *Rht8* and Xgwm 261-192 bp in Great Plains germplasm.

The objective of the second study was to determine the effects of allelic variation at the Xgwm 261, *Rht-B1*, and *Rht-D1* loci on GA sensitivity, coleoptile length, and agronomic traits of two winter wheat recombinant inbred line (RIL) populations. The two populations ['Longhorn'/'Akron' (LA), Longhorn/'Yuma' (LY)] were planted at five locations in eastern Colorado for two years and measurements for plant height, grain yield, test weight, kernel weight, kernel number, heading date, and above ground biomass were recorded. Microsatellite markers Xgwm 261 (*Rht8*), BF-MR1 (*Rht-B1*), and DF-MR2 (*Rht-D1*) were used to characterize the RIL populations. Gibberellic acid sensitivity and coleoptile length were evaluated in greenhouse and growth room conditions. Lower GA sensitivity and shorter coleoptile length was observed in RILs carrying the *Rht-B1b* allele compared with RILs carrying the *Rht-B1a* allele. Later heading date and taller plant height were consistently observed in RILs carrying the Xgwm 261-210 bp allele in combination with either *Rht-B1b* or *Rht-B1a*. Under the low yielding production conditions of the Great Plains region, the Xgwm 261-210 bp + *Rht-B1a* allelic class did not reduce grain yield and increased test weight.

Sally R. Clayshulte
Soil and Crop Sciences Department
Colorado State University
Fort Collins, CO 80523
Fall 2005

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Introduction

The semi-dwarfing genes *Rht-B1b* and *Rht-D1b* (previously known as *Rht1* and *Rht2*), derived from the Japanese variety 'Norin 10', have been used extensively in the development of semi-dwarf wheat (*Triticum aestivum* L.) cultivars. Plant height reduction improves straw strength under high input and irrigated conditions, resulting in more harvestable grain (Hoogendoorn et al., 1990; Evans, 1993; Börner et al., 1997). Additionally, yield is enhanced through increased partitioning of assimilates to the head instead of the stem (Youssefian et al., 1992a, b). These yield increases led to widespread adoption of the *Rht* semi-dwarfing genes by The International Maize and Wheat Improvement Center (CIMMYT) and other public breeding programs facilitating the Green Revolution in wheat (Hedden, 2003). However, since the introduction of *Rht-B1b* and *Rht-D1b* semi-dwarfing genes, yield has been variable under less than optimal conditions.

Another source of semi-dwarfism, *Rht8* from the variety 'Akakomugi', was introduced into European wheats in the 1930's by the Italian breeder Nazareno Strampelli. Exhibiting greater yield stability than *Rht-B1b* and *Rht-D1b* in certain Mediterranean countries, it is currently used to reduce heights of commercial wheat cultivars grown in southern and eastern Europe, Russia, China, and Japan (Borojevic, 1998; Worland et al., 1998; Rebetzke and Richards, 2000).

Based on plant height alone, determination of a specific *Rht* genotype is not possible. However, utilizing a gibberellic acid (GA) seedling test, semi-dwarfing genes can be separated into two groups: those that are sensitive to GA and those that are not (Allan et al., 1959; Worland et al., 1998). The semi-dwarfing genes *Rht-B1b* and *Rht-D1b* are GA insensitive resulting in a shorter coleoptile, smaller seedling leaf area, decreased internode length, and decreased plant height (Allan, 1989; Richards, 1992b; Beharev et al., 1998; Schillinger et al., 1998; Rebetzke and Richards, 2000). Coleoptile length is important for successful crop establishment in the low-precipitation dryland regions of the Great Plains, where deep planting is often necessary to place the seed into adequate soil moisture for germination (Stockton et al., 1996). Therefore, reduced coleoptile length is usually considered to be an unfavorable characteristic (Allan, 1980; Gale and Youssefian, 1985; Schillinger et al., 1998).

The semi-dwarfing gene *Rht8* confers sensitivity to exogenous GA similarly to the tall (*rht*) wheats. Therefore, it has been difficult to detect in segregating populations (Korzun et al., 1998; Worland, 1986) and to determine the extent to which this gene has been utilized. With the discovery of the microsatellite marker Xgwm 261, this difficulty has been alleviated (Korzun et al., 1998). Utilizing Xgwm 261 in two recombinant inbred line (RIL) populations, three alleles distinguishable by fragment sizes of 165 bp, 174 bp, and 192 bp were identified. Segregation of Xgwm 261-192 bp and *Rht8* revealed one recombinant genotype. Korzun et al. (1998) concluded that Xgwm 261-192 bp, with linkage of 0.6 cM to *Rht8,* may be useful as a diagnostic marker for *Rht8*.

Further analysis of entries originating from non-U.S. countries determined the Xgwm 261 locus to be highly polymorphic with three main allelic variants: 165 bp, 174 bp, and 192 bp (Korzun et al., 1998; Worland et al., 2001). In U.S. wheats, Ahmad and Sorrells (2002) utilized microsatellite marker Xgwm 261 to identify two main allelic variants: 165 bp, and 174 bp. Evaluating entries from the Great Plains region, Bai et al. (2004) utilized microsatellite marker Xgwm 261 along with coleoptile length measurements to determine the most common alleles among 80 hard winter wheat accessions. The 165 bp, 174 bp, and 192 bp fragments were the most common alleles identified. Though present in low frequency, Bai et al. (2004) concluded that the192 bp allele was scattered among hard winter wheat breeding programs in the Great Plains. However, accessions that contained the Xgwm 261-192 bp allele had no longer coleoptile length than those that did not (Bai et al., 2004).

Distinguishing between GA insensitive *Rht-B1b* and *Rht-D1b* alleles has not been readily accomplished until recently. Ellis et al. (2002) developed the BR-MR1 and DF-MR2 primer pairs that differentiate between the *Rht-B1b* and *Rht-D1b* and the *Rht-B1a* and *Rht-D1a* (wild type) alleles. Butler et al. (2005) utilized these microsatellite markers to identify the *Rht-B1* and *Rht-D1* alleles in a RIL spring wheat population. Plant height was found to be relatively consistent across four environments when correlated to the identified allelic classes. Flintham et al. (1997) and Butler et al. (2005) reported similar plant height reductions of 15.5% for *Rht-B1b* or *Rht-D1b*. However, Richards (1992) found greater plant height reductions (23%) for a single dwarfing gene emphasizing the importance of genetic background and environment in determining the extent of plant height reduction.

The use of molecular markers to identify the *Rht-B1*, *Rht-D1,* and *Rht8* allelic variants along with the determination of plant height, GA sensitivity, and coleoptile length offers plant breeders useful information as they develop new varieties of wheat for the Great Plains region. Planting varieties with greater coleoptile length is more likely to result in good stand establishment under a variety of planting conditions. Semi-dwarf plant height contributes to greater yield through reduced lodging and increased assimilates going to the grain. Taken together in a wheat variety, longer coleoptile length and semi-dwarf plant height offer the grower greater yield potential.

History of semi-dwarfing genes in wheat

Increases in wheat yield in the last four decades, following the 'Green Revolution', have been associated with the replacement of conventional tall cultivars with semi-dwarf ones (Hoogendoorn et al., 1990; Youssefian et al., 1992a, b; Evans, 1993; Börner et al., 1997; Korzun et al., 1998; Beharav et al., 1998; Hedden, 2003). The adoption of semi-dwarf varieties has been widespread. These varieties have stiff straw and are less prone to stem collapse or lodging before harvest. Severe lodging can make harvesting impossible with agricultural equipment and even moderate lodging can result in a decrease in final grain yield with inferior processing quality. The large yield increases after the adoption of semi-dwarf varieties have been attributed both to genetic improvement and to more intensive crop husbandry, particularly increased fertilizer use, which is permissible with these genotypes (Gale and Youssefian, 1985).

The development of semi-dwarf varieties of bread wheat is not a recent breeding objective. Breeders in Japan in the 19th century were probably the first to employ genetic sources of short stature wheat. A measure of their success is provided by the fact that most present-day short varieties of wheat owe their semi-dwarf characteristics to two Japanese genotypes (Gale and Youssefian, 1985).

The first of these was the variety 'Akakomugi', which was obtained by Nazareno Strampelli at Reiti in Italy in 1911. His objectives were to introduce early maturity and straw strength into Italian varieties. The varieties produced from his crosses with Akakomugi, for example 'Ardito' and later 'Mara', incorporated both of these target traits and have formed the backbone of Italian hexaploid wheat breeding. Although the inheritance of semi-dwarfism from Akakomugi was unknown, the genes involved were transferred to other European breeding programs (Gale and Youssefian, 1985).

The Italian wheats have also played an important part in the development of the short, 'daylength-insensitive' genotypes produced by the Rockefeller Foundation International Wheat Improvement Project, in Mexico, which in 1963 became CIMMYT. 'Mentana', which was one of Strampelli's taller varieties, was used in many breeding programs and is cited by Borlaug (1968) as one of the three key varieties in the Mexican program in the 1940s. Ardito, one of the shortest of Strampelli's varieties, has also been grown outside Italy and was employed in the parentage of the important Russian variety, 'Bezostaya' (FAO, 1971; Gale and Youssefian, 1985).

The second Japanese source of dwarfism was 'Daruma'. The initial crosses of 'Shirodaruma' (white) and 'Akadaruma' (red) with American varieties were made early in the 1900's in Japan (Gotoh, 1977). 'Norin 10' was one derivative of Shirodaruma. It was originally known as 'Tohuko No. 34', but was renamed in 1934 (Matsumoto, 1968). Norin 10 was not a significant variety in Japan, but was included among wheat germplasm sent to Dr. Orville Vogel at Washington State University in 1946. At the time, with the increasing use of nitrogen fertilizers, Vogel was looking for wheat germplasm with short plant height for use in the Pacific Northwest region of the U.S. He made the first crosses with Norin 10 in 1948 and produced Norin 10/Brevor 14. This genotype was to become the main source of the two semi-dwarfing genes *Rht-B1b* and *Rht-D1b* for both the U.S. and Mexican breeding programs. Other sources of *Rht-B1b* and *Rht-D1b* include 'Norin 16' and the Korean varieties 'Suweon 92' and 'Seu Seun 27', all of which originated from crosses with Daruma (Gale and Youssefian, 1985).

The spread of semi-dwarfing genes through the world wheat crop has been dramatic by plant breeding standards. Vogel released 'Gaines', the first variety to carry the Norin 10 semi-dwarf genes, in 1961 (Vogel, 1964). Gaines was followed by 'Nugaines' (Vogel and Peterson, 1974) in 1965 and more than 93 cultivars derived from Norin 10 had been released in North America by 1979 (Gale and Youssefian, 1985).

In Mexico, the use of Norin 10/Brevor 14 in crosses with tall, daylength insensitive wheats produced semi-dwarf varieties of great international significance. Borlaug made the first crosses with Norin 10/Brevor 14 in 1955 and the Mexican government wheat program released the varieties 'Pitic 62' and 'Penjamo 62' to farmers in 1962. Since then, all CIMMYT bread wheat lines have carried semi-dwarfing genes (Gale and Youssefian, 1985).

In addition to the semi-dwarfing genes available through the Japanese varieties Akakomugi and Daruma, there have been many attempts to induce useful, dwarfing genes by mutation breeding. A couple of the more promising ones are *Rht12* (from Karcagi 522 M7K) and *Rht13* (from Magnif 41 M1). *Rht12* is associated with a prostrate growth habit and late maturity (Konzak, 1984). Reduced plant height associated with *Rht13* is not evident until heading, when internode elongation appears to be reduced (Konzak, 1982). They have almost complete dominance for the semi-dwarfing plant height trait unlike many of the other semi-dwarfing genes developed from mutation breeding (Konzak, 1982, 1984). However, none of the induced plant height mutations have been used extensively in wheat breeding programs.

Genetic determination of semi-dwarfing genes

Although the genetics of plant height in wheat has been extensively studied, until recently relatively few genes had been genetically characterized. This is because of the quantitative nature of plant height and the polyploid nature of common wheat (Gale and Youssefian, 1985). In diploid crops, mutations are relatively easy to identify unlike in hexaploid wheat where deletions, along with recessive or null mutations, may not be readily observed because of the buffering provided by homoeologous alleles. Therefore, in hexaploid wheat, variation is observed only in cases of active (usually dominant) allelic variants or recessive mutants at unique untriplicated loci. Even then, allelic differences need to be large before they give rise to the discrete segregations necessary for conventional Mendelian analysis (Gale and Youssefian, 1985).

Genes affecting plant height were found to be located on several chromosomes through the use of monosomic analysis (Allan and Vogel, 1963). Utilizing coleoptile length analysis, Allan (1970) first suggested that two semi-dwarfing genes were involved in the dwarfism of Norin 10. Gale and Marshall (1976) located *Rht-B1b* on chromosome 4B while Gale et al. (1975) located *Rht-D1b* on chromosome 4D. Later McVittie et al. (1978) were able to position *Rht-B1b* 13 map units and *Rht-D1b* 15 map units from the centromere on the short arm of the chromosomes 4B and 4D, respectively. *Rht8* was located on chromosome 2D through the use of backcross monosomic analysis using ‘Koga II’ monosomics and ‘Sava’ (*Rht8*; Gale et al., 1982).

With the development of molecular markers, trait identification can be based on the presence of specific DNA sequences. Microsatellite markers consist of simple sequence repeats (SSRs) that can detect higher levels of polymorphism than other types of markers such as restriction fragment length polymorphism (RFLP; Henry, 1997). Additionally the repeatability of these markers makes them useful in discriminating closely related hexaploid wheat lines (Plaschke et al., 1995; Röder et al., 1995; Bryan et al., 1997). Röder et al. (1998) developed more than 250 microsatellite markers, genetically mapping over 200. Deletion lines were utilized to physically map microsatellite markers on the group 2 chromosomes. Korzun et al. (1998) utilized the Xgwm 261 marker to analyze two sets of RILs developed between Mara and 'Ciano 67' and 'Cappelle-Desprez'. Polymerase chain reaction (PCR) analysis utilizing Xgwm 261 revealed three alleles, distinguishable by fragment sizes of 192 bp, 174 bp and 165 bp. Their results revealed a 1:1 segregation for Xgwm 261, within the RILs developed between Mara (Xgwm 261-192 bp) and Cappelle-Desprez (Xgwm 261-174 bp), with 53 lines carrying the Xgwm 261-192 bp allele and 43 lines carrying the Xgwm 261-174 bp allele. Further analysis comparing these results with previous classification of the lines for *Rht8* detected a single recombinant between the two loci. Consequently, Korzun et al. (1998) concluded that Xgwm 261-192 bp, with linkage of 0.6 cM to *Rht8,* may be useful as a diagnostic marker.

The *Rht-B1b* and *Rht-D1b* semi-dwarfing genes are associated with a single base-pair change responsible for the semi-dwarf phenotype (Peng et al., 1999). Recently, Ellis et al. (2002) developed PCR-based markers that are specific for the *Rht-B1b* and *Rht-D1b* alleles. The PCR markers discriminated between the *Rht-B1b* and *Rht-D1b* alleles and

Rht-B1a and *Rht-D1a* (tall) alleles in a range of wheat cultivars of known *Rht* genotypes as well as doubled haploid lines from a cross of 'Sunco' (*Rht-B1b*) and 'Tasman' (*Rht-D1b*). Ellis et al. (2002) were able to locate the markers to the homoeologous regions of chromosomes 4B and 4D, consistent with earlier mapped positions for the *Rht-B1b* and *Rht-D1b* alleles.

Gibberellic acid insensitive semi-dwarfing genes

Reported semi-dwarfing genes identified in bread wheat include: *Rht-B1b* and *Rht-D1b*, *Rht8, Rht12*, and several less commonly known genes such as *Rht3-7,* 9, *11,* and *13-21* (McIntosh et al., 2003). Identifying specific semi-dwarf genotypes is not possible based only on plant height (Gale and Youssefian, 1985). *Rht-B1b* and *Rht-D1b* appear to have very similar effects on plant height and can be modified by background genotype. Early in the development of Norin 10 derivatives, Allan et al. (1959) noted that these lines differed from tall and other semi-dwarf genotypes in that their response to applied gibberellic acid (GA) did not result in increased stem elongation. Therefore, utilizing a GA seedling test, semi-dwarfing genes can be separated into two groups: those that are sensitive to GA and those that are not (Worland et al., 1998).

Initial reports indicated plant height and GA sensitivity were linked giving rise to a second series of gene symbols, *Gai1* and *Gai* (McIntosh, 1979). However, subsequent genetic and physiological evidence has indicated that the two phenotypes are pleiotropic effects of the same gene (Gale and Marshall, 1976).

Characteristics of *Rht-B1b* and *Rht1-D1b* alleles

Wheats that carry the *Rht-B1* and *Rht-D1* genotypes, excluding the tall alleles *Rht-B1a* and *Rht-D1a*, are insensitive to GA (Rebetzke and Richards, 2000). The *Rht-B1* and *Rht-D1* mutations are associated with a single base-pair change leading to a TAG stop codon shortly after the start of translation (Peng et al., 1999). These mutations alter the plant's ability to respond to GA and therefore exogenous GA application to juvenile plants does not restore wild-type (tall) plant height. Conversely, cells in juvenile tissues of GA sensitive lines elongate after application of exogenous GA. GA insensitivity found in the *Rht-B1* and *Rht-D1* genotypes indirectly affects the plant in numerous ways.

GA insensitivity does not appear to alter the timing of plant development, compared with GA sensitive genotypes with similar dates of emergence, final numbers of leaves, and internodes (Borrell et al., 1991; Youssefian et al., 1992b; Miralles and Slafer, 1995; Calderini et al., 1996). However, growth analysis indicates that the *Rht-B1b* and *Rht-D1b* alleles affect extension growth in the stems and leaves (Hoogendoorn et al., 1990), resulting in smaller seedling leaf areas, shorter internode length, culm length, coleoptile length and plant height (Nilson et al., 1957; Allan, 1989; Pinthus et al., 1989; Borrell et al., 1991; Richards, 1992b; McCaig and Morgan 1993; Beharev et al., 1998; Schillinger et al., 1998; Rebetzke and Richards, 2000). The smaller leaf size and internode length is due to changes in cell size and not the duration of growth period or cell number (Gale et al., 1985; Keyes et al., 1989; Borrell et al., 1991; Calderini et al., 1996). Reduced cell size was found to be associated with reduced osmotic potential of the cell solutes (Hoogendoorn et al., 1990).

The *Rht-B1b* and *Rht-D1b* alleles are found to reduce coleoptile length in similar proportions to their effects on plant height (Allan, 1970). As with plant height, the background genotype can modify the effects of the *Rht-B1b* and *Rht-D1b* alleles on coleoptile length and final stand establishment (Allan, 1980). Coleoptile length is important for successful crop establishment in the low-precipitation dryland regions of the Great Plains, where deep planting is often necessary to place the seed into adequate soil moisture for germination (Budak et al., 1995; Schillinger et al., 1998). Therefore, reduced coleoptile length is usually considered to be an unfavorable trait, associated with reduced seedling emergence and subsequent poor stand establishment unless sowing depth can be adequately reduced (Allan, 1980; Gale and Youssefian, 1985).

When water or nutrient supplies are limiting, the extent of the root system, and particularly the depth of rooting, may be critical. Genotypes with shallow roots may display increased sensitivity to drought stress conditions. Root weights and root depth were closely related to shoot weight and plant height among isogenic lines and varieties with *Rht-B1b* and *Rht-D1b* (Virmani, 1971; MacKey, 1973). In contrast, Welbank and Taylor (1971) found dwarf varieties had larger root systems while Cholick et al. (1977) indicated no consistent differences in root development between semi-dwarf and tall wheats.

Varietal comparisons have confirmed enhanced yielding ability of varieties carrying *Rht-B1b* and *Rht-D1b* in India (Kulshrestha and Jain, 1979), Mexico (Borlaug, 1968), Australia (Cooper, 1979), the U.K. (Austin et al., 1980) and the U.S. (Austin et al., 1980; Allan, 1989). Shorter plant height has resulted in improved lodging resistance (Börner et al., 1997). Additionally, the growth of the wheat spike was found to be indirectly affected in GA insensitive genotypes including reduced competition from the stem thereby increasing the partitioning of assimilates to the developing spike allowing the spike to attain greater mass at anthesis (Youssefian et al., 1992a,b). The *Rht-B1b* and *Rht-D1b* alleles promote more fertile florets per spikelet resulting in an increase in the number of grains per spike, although a compensative decrease in kernel weight occurs under most environmental conditions (Gale, 1979; Allan, 1989; Fischer and Quail, 1990; Richards, 1992a; Börner et al., 1997; Beharav et al., 1998). However, Borrell et al. (1991) were unable to determine if the increased partitioning of assimilates to the spikes and accompanying higher number of grains per spike was due to *Rht-B1b* and *Rht-D1b* or simply to a reduction in plant height.

Yield reductions associated with *Rht-B1b* and *Rht-D1b*

Examination of national yield data and climatic fluctuations indicates that wheat yields are more sensitive to environmental variation in countries that have adopted the Norin 10 semi-dwarf alleles than in areas where *rht* (tall) genotypes are still prominent (Michaels, 1982). It may be that the *Rht-B1b* and *Rht-D1b* genotypes are more stress sensitive so that under unfavorable conditions they are unable to realize their high-yielding potential, resulting in less stable yields (Gale and Youssefian, 1985). Wheats carrying the *Rht-B1b* and *Rht-D1b* alleles also have been associated with yield reductions in low yielding environments (Kertesz et al., 1991; Rebetzke and Richards, 2000). Reduced yield might arise from lower biomass and water use efficiency where water is limiting (Richards, 1992b) or from poor establishment due to shorter coleoptiles and reduced early vigor (Rebetzke and Richards, 2000). The germination, emergence, and establishment phases are critical to the growth cycle of wheat as they determine the density of the stand obtained, influence the degree of weed infestation, and the yield potential. Germination and crop establishment are especially critical in the arid regions where germinating seeds may be subjected to variable moisture levels. This problem can be exacerbated when sowing wheat varieties carrying the *Rht-B1b* and *Rht-D1b* alleles with their associated short coleoptiles (Schillinger et al., 1998) .

When soil moisture is limiting, deep sowing may allow growers to make use of soil moisture lying below the drying topsoil so that sowing can occur at the optimal time (Schillinger et al., 1998). Deeper sowing also provides an option for avoiding the harmful effects of pre-emergent herbicides. Additionally, seed may be sown deep unintentionally when depth control on the seeder is poor or when planting on uneven ground. High soil temperatures along with a reduction in coleoptile length results in decreased seedling emergence (Fick and Qualset, 1976; Radford, 1987). Wheat seed planted 5 cm deep in an irrigated off-season summer nursery had a seedling emergence of 50% compared to 80-90% for wheat planted during the normal fall season (Fick and Qualset, 1976). Wheat stands can also be reduced where stubble or a hard seed-bed restrict the growth of the coleoptile through the soil surface (Rebetzke and Richards, 2000).

In environments where stress due to drought or high temperatures occurs, yield of varieties carrying the *Rht-B1b* and *Rht-D1b* alleles is often lower than varieties with the *rht* (tall) wheats (Richards, 1992a; Miralles and Slafer, 1995). Additionally, under certain circumstances where warm temperatures occur around the time of meiosis, interactions between the dwarfing genes *Rht-B1b* and *Rht-D1b* and the environment can cause fertility reductions and loss of yield advantages (Worland et al., 1998). Furthermore, at higher production levels, throughout the Australian wheat belt when there is a post-anthesis drought, varieties with *Rht-B1b* or *Rht-D1b* often have greater yield losses than the *rht* varieties. Similarly, there is a high probability for this type of post-anthesis drought condition in the Great Plains region (Richards, 1992b).

Utilizing molecular markers to identify *Rht-B1b* and *Rht-D1b*

The presence of gibberellic acid insensitive *Rht-B1b* and *Rht-D1b* alleles can be determined by testing seedlings for their response to GA (Richards, 1992a). However, this method does not distinguish between the two alleles. Recently, Ellis et al. (2002) developed PCR-based microsatellite markers that differentiate between the *Rht-B1b* and *Rht-D1b* and the *Rht-B1a* and *Rht-D1a* (wild type) alleles. The BR-MR1 and DF-MR2 primer pairs are specific for the base pair change responsible for reduced plant height at the *Rht-B1* and *Rht-D1* loci. Utilizing the BR-MR1 and DF-MR2 primer pairs, entries from Mexico, Australia, Canada, and Japan along with a doubled haploid population of Sunco (*Rht-B1b*) x Tasman (*Rht-D1b*) were differentiated for the *Rht-B1b* and *Rht-D1b* alleles (Ellis et al., 2002).

Butler et al. (2005) utilized the BR-MR1 and DF-MR2 primer pairs to identify alleles at the *Rht-B1* and *Rht-D1* loci in a RIL spring wheat population. At both the *Rht-B1* and *Rht-D1* loci, there were plant height reductions confirming the presence of *Rht-B1b* and *Rht-D1b.* The two alleles together accounted for 59% of the phenotypic variance for plant height. Smaller height reductions compared with those found by Richards (1992a) confirmed the importance of genetic background and environment in determining the magnitude of height reduction.

Rht8

Wheat breeders in southern Europe were interested in improving the adaptability of their varieties as well, by reducing plant height, and increasing spikelet fertility and thereby enhancing yield (Worland et al., 1998). Introducing *Rht-B1b* and *Rht-D1b* for shorter plant height and greater spikelet fertility was one option (Gale and Youssefian, 1985; Worland et al., 1998). However, the *Rht-B1b* and *Rht-D1b* alleles derived from the variety Norin10 are not universally beneficial and were found to reduce rather than promote improvement in plant performance in Mediterranean countries (Ahmad and Sorrells, 2002). In fact, under certain circumstances where warm temperatures occur around the time of meiosis, interactions between these dwarfing genes and the environment can cause fertility reductions and loss of yield (Law and Worland, 1985).

In these countries, a different source of semi-dwarfism, derived from the variety Akakomugi has been utilized. The Italian breeder Nazareno Strampelli obtained Akakomugi in 1911 and in the 1930's introduced *Ppdl* and *Rht8* into the European wheats. *Ppdl* confers photoperiod insensitivity and indirectly reduces plant height by shortening the plant life cycle (Worland, 1996). Promoting a reduction in plant height by accelerating the number of days to flowering restricts the use of *Ppdl* to environments where earlier flowering can be tolerated. The semi-dwarfing gene *Rht*8 confers shorter plant height similar to *Rht-B1b* and *Rht-D1b* and has been used commercially to reduce heights of wheat varieties grown in southern and eastern Europe, Russia, China, and Japan (Worland et al., 1998; Rebetzke and Richards, 2000).

Data from RILs from a Mara (*Rht8*) x Cappelle-Desprez (*rht8*) population grown in several environments suggests that *Rht8* reduces plant height by 8-10 cm in the U.K. (Worland et al., 1988a,b), 5 cm in Germany (Worland et al., 1992), and 5-7 cm in Yugoslavia (Worland et al., 1988a,b). Additionally, no adverse effects of *Rht8* on plant growth, coleoptile length, and yield have been detected (Worland et al., 1998). In fact, Gale et al., (1982) found 12 % greater yield on primary tillers of lines carrying *Rht8*. Their results suggested that lines carrying *Rht8* may offer the advantages of semi-dwarf plant height for lodging resistance and increased partitioning of assimilates to the grain without the adverse effects of reduced coleoptile length and variable yields under high heat and drought stress observed when utilizing *Rht-B1b* and *Rht-D1b* for reduced plant height. Therefore, one strategy to achieve adult-plant height reduction without the negative consequences of reduced coleoptile length may be to introgress *Rht8* into wheat breeding programs (Bai et al., 2004).

Genetic determination of *Rht8*

The inheritance of Akakomugi dwarfism was characterized more than 70 years after its first use in Italy. *Rht8* does not confer insensitivity to exogenous GA, unlike *Rht-B1b* and *Rht-D1b*, and therefore is not easily identified in segregating populations. Gale et al. (1982) utilized the Koga II monosomics to determine that *Rht8* was a single dwarfing gene located on chromosome 2D and accounted for almost all the shorter plant height conferred by the semi-dwarfing gene from Akakomugi. Additionally, an intervarietal chromosome substitution series was developed for the variety Mara in a Cappelle-Desprez background (Law et al., 1978). Confirming earlier work, analysis of plant height in these lines showed a major height-reducing factor on chromosome 2D (Law et al., 1978).

The presence of *Rht8* in a variety can only be unequivocally be established by the use of a monosomic for the 2D chromosome that carries the gene (Worland et al., 1998). Observations on height of group 2 monosomics compared to that of the parental variety have indicated that the varieties Mara, Sava, and 'NS Rana' carry *Rht8* on their 2D chromosome. The presence of the *Rht8* in other varieties could only be identified by carrying out reciprocal monosomic analysis between the variety and established group 2 monosomics (Snape and Law, 1980).

The sensitivity of *Rht8* to GA has made it difficult to detect in segregating populations (Worland, 1986; Korzun et al., 1998). With the discovery of microsatellite marker Xgwm 261, this problem has been eased (Korzun et al., 1998). PCR analysis, of 'Mara', 'Cappelle-Desprez' and 'Ciano 67' in two RIL populations utilizing Xgwm 261, identified three alleles distinguishable by fragment sizes of 192 bp, 174 bp and 165 bp. Segregation of Xgwm 261-192 and *Rht8* revealed one recombinant genotype. Korzun et al. (1998) concluded that Xgwm 261-192 bp, with linkage of 0.6 cM to *Rht8,* may be useful as a diagnostic marker. Further analysis of over 100 lines from non-U.S. origins determined the Xgwm 261 locus to be highly polymorphic with three main allelic variants: 165 bp, 174 bp, or 192 bp (Korzun et al., 1998).

Further screening of over 870 entries from 20 countries, including eight from the U.S., Worland et al. (2001) revealed ninety percent of the entries carried the 165 bp, 174 bp, or 192 bp allele with the other 10% carrying allelic variants with fragments of 195, 196, 197, 201, 202, 203, 204, 205, 207, 210, or 215 bp. The varieties identified with the Xgwm 261-192 bp allele included many that were known to carry the Akakomugi donor of *Rht8* from southern Europe. Ninety-four percent of the screened CIMMYT varieties carried the Xgwm 261-165 bp allele. Differences in height, similar to Korzun et al. (1998), were found between the 165 bp, 174 bp and 192 bp allelic classes (Worland et al., 2001). The varieties Mara, NS Rana and Sava, previously determined to carry *Rht*8, all carried the WMS 261-192 bp microsatellite allele. Conversely, the Xgwm 261-192 bp allele was not detected in control varieties where *Rht*8 would not be expected by pedigree analysis or by monosomic comparisons (Worland et al., 1998).

Chebotar et al. (2001) performed additional screening, utilizing microsatellite Xgwm 261, evaluating 27 entries from the Ukrainian Research Institutes and 20 non-Ukrainian entries, including seven U.S. entries. Eighty-eight percent of the Ukrainian entries carried the 192 bp allele indicating the introgression of *Rht8* as an alternative semi-dwarfing source (Chebotar et al., 2001) to the *Rht-B1b* and *Rht-D1b* alleles. The

Rht-B1b and *Rht-D1b* alleles proved to have a negative association with yield under Ukrainian climatic conditions (Kertesz et al., 1991; Chebotar et al., 2001).

In the first study to focus on U.S. wheats, Ahmad and Sorrells (2002), screened 41 entries from the U.S., including four Hard Red Winter wheats from the Great Plains region, along with 30 additional entries from 12 countries. Their objective was to evaluate allelic variation at the *Rht8* locus, utilizing microsatellite marker Xgwm 261, in a wide range of U.S. and New Zealand wheat entries. They determined that the U.S. entries carried three main allelic variants: 165 bp, 174 bp, and 204 bp.

Utilizing genotypes from the Great Plains region, Bai et al. (2004) surveyed 80 hard winter wheat entries with microsatellite marker Xgwm 261 and for coleoptile length measurements. Their results corresponded with previously screened international wheat entries (Korzun et al., 1998; Worland et al., 1998, 2001; Ahmad and Sorrells, 2002), with the most common alleles of the hard wheat entries being the 165 bp, 174 bp, and 192 bp fragments. Other alleles identified among the entries included 195, 196, 197, 201, 203, 205, 210, and 215 bp fragments. Though present in low frequency, Bai et al. (2004) concluded that the 192 bp allele was scattered among hard winter wheat breeding programs in the Great Plains. However, on the basis of the available pedigrees, they were unable to confirm the presence of *Rht8* in the U.S. accessions since the 192 bp allele is a marker linked to *Rht*8 and not part of the gene itself (Bai et al., 2004). The presence of the 192 bp allele did not appear to be associated with greater coleoptile length. Therefore, Bai et al. (2004) concluded that the presence of the 192 bp allele does not ensure increased coleoptile elongation in a semi-dwarf plant type.

Although numerous studies have evaluated non-U.S. entries for distribution patterns of the Xgwm 261-192 bp allele, only Bai et al. (2004) evaluated a substantial number of hard winter wheat entries. Furthermore, to date no study has characterized hard winter wheat entries to identify allele variants at the Xgwm 261, *Rht-B1*, and *Rht-D1* loci and evaluate the effects of these semi-dwarfing genes on plant height, GA sensitivity, and coleoptile length along with the agronomic traits of yield, test weight, heading date, above ground biomass, kernel weight and kernel number. A better understanding of the allelic effects of *Rht8*, *Rht-B1b,* and *Rht-D1b* will aid plant breeders in selection of superior genotypes for reduced plant height and increased productivity for specific wheat production conditions.

CHAPTER ONE

Trait Associations and Allelic Diversity at the Xgwm 261, *Rht-B1* and *Rht-D1* Loci in Historical and Current Great Plains Entries.

ABSTRACT

The semi-dwarfing genes *Rht-B1b* and *Rht-D1b* have been used extensively in the development of reduced height wheat (*Triticum aestivum* L.) cultivars. Yield increases have been observed through decreased lodging and improved partitioning of assimilates, although reduced stand from shorter coleoptiles has been cited as a disadvantage. Interest in the gibberellic acid sensitive semi-dwarfing gene *Rht8* has increased due to the discovery of Xgwm 261, a closely linked microsatellite marker, which may be useful as a diagnostic marker for *Rht8*. The objectives of this study were to determine allelic variation at the Xgwm 261 marker locus among historical and current Great Plains hard winter wheat germplasm, identify alleles at the *Rht-B1* and *Rht-D1* loci, and evaluate the pleiotropic effects of these semi-dwarfing genes on plant height, gibberellic acid sensitivity, and coleoptile length. Microsatellite markers Xgwm 261, BF-MR1 and DF-MR2 were used to determine the allelic identity at the *Rht8*, *Rht-B1* and *Rht-D1* loci. Plant height, gibberellic acid sensitivity and coleoptile length were evaluated in greenhouse and growth room conditions. Four allelic classes were identified among 72 Great Plains entries with fragment sizes of 165 bp, 174 bp, 192 bp, and 210 bp. *Rht-B1b*, *Rht-D1b,* and *rht* (tall) genotypes were identified among Xgwm 261 allelic classes. Variation across Xgwm 261 allelic classes ranged from 84.2 to 97.1 cm for plant height,

5.0 to 13.9 cm for gibberellic acid sensitivity, and 8.4 to 9.4 cm for coleoptile length. *Rht* versus *rht* alleles were associated with a 10.2 cm increase in plant height, 15.2 cm increase of gibberellic acid response, and 1.4 cm increase of coleoptile length across the four allelic classes of Xgwm 261. Among Great Plains winter wheat germplasm, this study was unable to confirm the diagnostic capability of the Xgwm 261 microsatellite marker to identify germplasm with reduced height, gibberellic acid sensitivity, and longer coleoptiles. This suggests the lack of linkage between *Rht8* and Xgwm 261-192 bp in the Great Plains germplasm.

INTRODUCTION

The semi-dwarfing genes *Rht-B1b* and *Rht-D1b* (previously known as *Rht1* and *Rht2*), derived from the Japanese variety 'Norin 10', have been used extensively in the development of semi-dwarf wheat cultivars. Plant height reduction improves straw strength under high input and irrigated conditions, resulting in more harvestable grain (Hoogendoorn et al., 1990; Evans, 1993; Börner et al., 1997). Additionally, yield is increased through increased partitioning of assimilates to the head instead of the stem (Youssefian et al., 1992a, b). These yield increases led to widespread adoption of the *Rht* semi-dwarfing genes by CIMMYT and other public breeding programs, facilitating the Green Revolution in wheat (Hedden, 2003). However, since the introduction of the *Rht-B1b* and *Rht-D1b* semi-dwarfing genes, yield has been variable under less than optimal conditions.

Another source of semi-dwarfism, *Rht8* from the variety 'Akakomugi', was introduced into European wheats in the 1930's. Exhibiting greater yield stability than *Rht-B1b* and *Rht-D1b* in certain Mediterranean countries, *Rht8* is currently used to reduce heights of commercial wheat cultivars grown in southern and eastern Europe, Russia, China, and Japan (Worland and Law, 1986; Borojevic, 1998; Worland et al., 1998; Rebetzke and Richards, 2000).

Reported *Rht* genes identified in bread wheat include: *Rht-B1b*, *Rht-D1b*, *Rht8*, and several less commonly known genes such as *Rht3-7, 9*, and *11-21* (McIntosh et al., 2003). Based on plant height alone, determination of a specific *Rht* genotype is not possible. However, utilizing a gibberellic acid (GA) seedling test, *Rht* genes can be separated into two groups: those that are sensitive to GA and those that are not (Worland et al., 1998). Gibberellic acid insensitivity affects the plant pleiotropically, resulting in shorter coleoptiles, smaller seedling leaf area, decreased internode length, and decreased plant height (Allan, 1989; Keyes and Paolillo, 1989; Beharev et al., 1998; Miralles et al., 1998). Coleoptile length is important for successful crop establishment in the low-precipitation dryland regions of the Great Plains, where deep planting is often necessary to place the seed into adequate soil moisture for germination (Stockton et al., 1996). Therefore reduced coleoptile length is usually considered to be an unfavorable characteristic (Allan, 1980; Gale and Youssefian, 1985; Schillinger et al., 1998).

Rht8 alleles confer sensitivity to exogenous gibberellic acid similar to tall (*rht*) alleles and therefore have been difficult to detect in segregating populations (Worland, 1986; Korzun et al., 1998). With the discovery of microsatellite marker Xgwm 261, this problem has been alleviated (Korzun et al., 1998). PCR analysis of 'Mara', 'Cappelle-Desprez', and 'Ciano 67' in two recombinant inbred line (RIL) populations utilizing Xgwm 261 identified three alleles distinguishable by fragment sizes of 192 bp, 174 bp and 165 bp. Co-segregation of Xgwm 261-192 bp allele and *Rht8* was not absolute with one recombinant individual identified. Korzun et al. (1998) therefore concluded that the Xgwm 261-192 bp allele, with linkage of 0.6 cM to *Rht8,* may be useful as a diagnostic marker for *Rht8*. Further analysis of over 100 lines originating from other areas of the world determined the Xgwm 261 locus to be highly polymorphic with three main allelic variants: 165 bp, 174 bp, and 192 bp (Korzun et al., 1998).

Further screening of over 870 entries from 20 countries, including eight from the U.S., Worland et al. (1998) revealed allelic variants to be distributed non-randomly for specific environments. Ninety percent of the entries carried the 165 bp, 174 bp, or 192 bp allele while the other 10% carried allelic variants with fragment sizes of 195, 196, 197, 201, 202, 203, 204, 205, 207, 210, or 215 bp. Chebotar et al. (2001) performed additional screening with microsatellite marker, Xgwm 261, evaluating 27 entries from the Ukrainian Research Institutes and 20 non-Ukrainian entries including seven from the U.S.. Eighty-eight percent of the Ukrainian entries carried the 192 bp allele.

In the first study to focus on U.S. wheats, Ahmad and Sorrells (2002) screened 41 entries from the U.S., including four Hard Red Winter (HRW) wheats from the Great Plains region along with 30 additional entries from 12 countries. Their objective was to evaluate allelic variation at the *Rht8* locus, utilizing microsatellite marker Xgwm 261, in a wide range of U.S. and New Zealand wheat germplasm. They determined that the U.S. entries had three main allelic variants: 165 bp, 174 bp, and 204 bp. Utilizing genotypes from the Great Plains region, Bai et al. (2004) evaluated 80 entries for microsatellite marker Xgwm 261 and coleoptile length. Their results corresponded with previous results (Korzun et al., 1998; Worland et al., 1998, 2001; Ahmad and Sorrells, 2002), with the most common alleles of the HRW wheat entries the165 bp, 174 bp, and 192 bp fragments. Other alleles identified among the Great Plains entries included 195, 196, 197, 201, 203, 205, 210, and 215 bp fragments. Though present in low frequency, Bai et al. (2004) concluded that the 192 bp allele was scattered among hard winter wheat breeding programs in the Great Plains. However, on the basis of the available pedigrees, they were unable to affirm the presence of *Rht8* in the U.S. accessions since the 192 bp allele is a marker linked to *Rht*8 and not part of the gene itself (Bai et al., 2004). Additionally, association between the presence of the 192 bp allele and greater coleoptile length could not be detected and Bai et al. (2004) concluded that the presence of the 192 bp allele does not ensure increased coleoptile elongation in a semi-dwarf plant type. Distinguishing between GA insensitive *Rht-B1b* and *Rht-D1b* alleles has not been possible until recently. Ellis et al. (2002) developed BR-MR1 and DF-MR2 primer pairs that differentiate between the *Rht-B1b/Rht-D1b* and the *Rht-B1a/Rht-D1a* (wild type) alleles. Butler et al. (2005) utilized the PCR-based microsatellite markers developed by Ellis et al. (2002) to identify alleles at the *Rht-B1* and *Rht-D1* loci in a spring wheat RIL population. Plant height was found to be relatively consistent across four environments when correlated to the identified allelic classes. Flintham et al. (1997) and Butler et al. (2004) reported similar plant height reductions of 15.5% for *Rht-B1b* or *Rht-D1b*. However, Richards (1992) found greater plant height reductions (23%) than Butler et al. (2004) emphasizing the importance of genetic background and environment in determining the extent of plant height reduction.

Although numerous studies have evaluated non-U.S. germplasm for distribution patterns of the Xgwm261-192 bp allele, only Bai et al. (2004) evaluated a substantial number of hard winter wheat entries from the U.S. Great Plains region. Furthermore, to date no study has characterized hard winter wheat entries to identify allelic variants at the Xgwm 261 locus along with alleles at the *Rht-B1* and *Rht-D1* loci. The objective of this study was therefore to determine allelic variation at the Xgwm261 marker locus among historical and current Great Plains HRW germplasm, identify alleles at the *Rht-B1* and *Rht-D1* loci, and evaluate the pleiotropic effects of these semi-dwarfing genes on plant height, gibberellic acid sensitivity, and coleoptile length.

MATERIAL AND METHODS

Plant Materials

The study included a set of eighty-nine entries; 72 were current and historical Great Plains entries, three were historical entries from the Pacific Northwest, and 14 were non-U.S. entries. Selection of Great Plains entries was based on their past and present commercial importance to the Great Plains region and included entries from Colorado, Kansas, Nebraska, Oklahoma, Texas, AgriPro Inc. and Westbred Inc. Several entries from other countries that reportedly carry *Rht8* were also included. Seed was obtained from the USDA-ARS National Small Grains Collection (NSGC) or individual wheat breeding programs.

Molecular Marker Analysis

DNA was isolated from bulked leaves of five to six seedlings of each entry according to the procedures of Riede and Anderson (1996). DNA was further purified with an addition of 1 μL RNAase and incubated at 37 °C for 30 m. Microsatellite marker Xgwm 261 was amplified in a 20 μL PCR reaction containing 80 ng DNA, 1x PCR buffer, 120 μ*M* dNTP, 2.5 μM of each Xgwm 261 primer, and 0.8 units of *Taq* polymerase. The following touchdown thermal profile was used for DNA amplification: 3 min at 94 °C, 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C for five cycles, in which the annealing temperature was lowered by 1 °C per cycle; and 31 cycles in which the annealing temperature remained constant at 50 °C. Ten minutes at 72 °C was used for the final extension. Amplified DNA was loaded into a polyacrylamide gel and electrophoresed at 70 W for 2 h and 30 min. Polyacrilimide gels were silver stained and scanned on a Microtek flat bed scanner (Microtek Int. Inc., Carson, CA) at 150 dpi resolution. Molecular sizes of the DNA fragments were determined by comparison with a 10 bp DNA ladder (Invitrogen, Inc., Carlsbad, CA) and comparison with three entries 'Yuma' (Xgwm 261-165 bp), 'Longhorn' (Xgwm 261-210 bp) and 'Mara' (Xgwm 261-192 bp).

Primer pairs developed by Ellis et al. (2002) were utilized to determine *Rht-B1* and *Rht-D1* alleles for the entries. Identification of *Rht-Bl* alleles was based on the primer pair designated BF-MR1 and identification of *Rht-D1* alleles was based on the DF-MR2 primer pair. The PCR conditions were the same as specified by Ellis et al. (2002), except that the annealing temperature for the BF-MR1 primer pair was increased to 62.4 °C. PCR products were loaded into 4% high resolution agarose gels (SFRA, Amresco, Solon, OH) and electrophoresed at 100 V for 2 h 30 min Agarose gels were stained with ethidium bromide for 30 min and rinsed for 30 sec in deionized water, then digitally photographed under ultraviolet light using the Alphaimager documentation system (Alpha Innotech Corp., San Leandro, CA). Molecular sizes of the DNA fragments were determined in relation to the Amplisize Molecular Ruler (BioRad, Richmond, CA) and comparison with two entries: Yuma (*Rht-B1b*) and Longhorn (*Rht-B1a*).

Height Measurement

Fifteen seeds of each entry were placed on a 5.08 cm x 10.16 cm steel blue blotter paper (Anchor Paper Co., St. Paul, MN) within 7.6 cm x 12.7 cm ziplock bags (Anchor Paper Co., St. Paul, MN). Five ml of 0.001 L, 10% carboxin/10% thiram liter^{-1} (Gustafson, LLC., Plano, TX) solution was added and the seeds were alternately placed for 48 h at room temperature and at 2 °C until germination occurred. Ziplocks with germinated seed were placed in a 2 °C cold room for eight wks for vernalization. After eight wks, four uniform seedlings were planted in each of three one-gallon plastic pots (American Clay Works, Denver, CO) filled with Metro Mix 350 (American Clay Works, Denver, CO). Pots were placed in the greenhouse and fertilized with 20 gm of osmocote (Scotts Sierra Horticultural Products Co., Marysville, OH.) with N-P-K equal to 14-14-14. At the three- leaf stage, one plant was removed from each pot. For insect control, 0.1 g of one percent granular Imidacloprid (Olympic Horticultural Products Co., Davenport, FL) was applied to each pot. At maturity, plants were measured from the soil level to the top of the heads, excluding awns. The experimental design was a randomized complete block with three replications. Seed produced in the plant height evaluation was used for gibberellic acid sensitivity, coleoptile length, and molecular marker analyses.

Gibberellic Acid Sensitivity Measurement

Plastic 38.1 x 15.3 x 5.1 cm trays (Rubbermaid Co., Wooster, OH) were filled 3/4 full with coarse vermiculite (American Clay Works, Denver, CO). Trays were planted with ten test entries and two check entries (Mara and Yuma) in each tray. Ten seeds of each entry were planted in a single row within the tray and covered with a layer of vermiculite. Trays were placed in the greenhouse and watered, either with distilled water or 25 ppm GA solution (Sigma-Aldrich Inc, St. Louis, MO), as necessary. After 21 d, plant height was measured from the seed to the tip of the tallest leaf. GA sensitivity was determined as the mean value of the 10 seedlings treated with distilled water subtracted from the mean value of the 10 seedlings treated with the GA_3 solution. The experimental design was a randomized complete block with two replications. Replications were temporally blocked due to a six month separation between replications.

Coleoptile Length Measurement

Forty seeds, embryo side down, were placed approximately 1 cm apart and 2.2 cm from the bottom on pre-moistened 39.6 x 13.2 cm blotter paper (Anchor Paper Co., St. Paul, MN). A pre-moistened blotter paper was placed over the seeds and both sheets were loosely rolled and secured with a size no. 32 rubber band (S.P. Richards Co., Atlanta, GA). Samples were placed in a plastic 43.8 x 29.5 x 16.5 cm container (Rubber Maid Co., Wooster, OH) and filled to a depth of 2 cm with distilled water. Containers were covered with foil and placed in a dark cold room at 4 °C for four d. Samples were then incubated in a dark Percival growth chamber (Percival Scientific, Inc., Boone, IA) at 16 °C for 16 d. Measurements were taken from the seed to the top of the coleoptile. The experimental design was a randomized complete block with two replications. Replications were temporally blocked due to a one month separation between replications.

Data Analysis

Analysis of variance and mean separations for plant height, GA sensitivity, and coleoptile length within each Xgwm 261 allelic class were performed utilizing SAS PROC ANOVA (LSD, SAS Institute Inc., 1999) at $\alpha = 0.05$. Fisher's least significance difference was used for mean separation. Utilizing SAS PROC GLM (SAS Institute Inc., 1999), least square mean comparisons for plant height, gibberellic sensitivity, and coleoptile length among the Xgwm 261 allelic classes and *Rht-B1* + *Rht-D1* loci were performed. Analysis of the interaction was not performed among Xgwm 261, *Rht-B1*, and *Rht-D1* allelic classes due to unbalanced samples sizes.

RESULTS AND DISCUSSION

Allelic Variation at the Xgwm 261 Locus

Microsatellite marker Xgwm 261 identified the 165 bp, 174 bp, 192 bp, and 210 bp allelic classes among the 89 entries evaluated (Table 1-1). The 165 bp fragment was identified in more entries (32) than any other allele, while the 174 bp fragment was identified in the fewest number of entries (6). Among the Great Plains entries, the 165 bp alleles was identified in 28 entries, the 174 bp allele in one entry, the 192 bp allele in 11 entries, and the 210 bp allele in 29 entries. Two entries, 'Avalanche' and 'Buckskin', amplified both the 165 bp and 210 bp alleles, suggesting heterogeneity within these entries.

The165 bp, 174 bp, and 192 bp Xgwm 261 alleles were the main variants identified in previous research (Korzun et al., 1998; Worland et al.; 1998, 2001; Bai et al., 2004). Worland et al. (1998) reported a 10 cm increase in plant height associated with the 165 bp allele and a 8 cm increase associated with the 174 bp allele. The 165 bp allele has been associated with CIMMYT-derived semi-dwarf wheat germplasm (Worland et al., 1998, 2001). Additionally, Ahmad and Sorrells (2002) proposed the U.S. HRW, region with its shorter and drier growing season, may favor the 165 bp allele with its taller plant height. The 174 bp allele with its shorter plant height may be preferred in the eastern U.S. with its longer growing season and greater soil moisture. In this study, the major allelic variants were the 165 bp, 192 bp, and 210 bp fragments. The 210 bp allele, identified by Worland et al. (1998), is found infrequently in European entries. However, Bai et al. (2004) identified the 210 bp allele in 27% of the 80 hard winter wheat entries evaluated. In this study, 41% of the Great Plains entries carried the 210 bp

Table 1-1. Origin, year of release, phenotypic values and molecular market classes for eighty-nine wheat entries evaluated.

Entry	Origin†	Year	Plant height	GA sensitivity	Coleoptile length	Xgwm 261	*Rht-B1b*‡	*Rht-B1a*	*Rht-D1b*	*Rht-D1a*
			cm							
2137	KSU-Manhattan	1995	88	2.7	7.4	165	A	P	P	P
2157	Pioneer-KSU. OSU, TX	1984	75.4	2.4	8	165	P	A	A	P
2174	OSU	1997	85.6	2	8	165	P	A	A	P
Abilene	AgriPro	1987	67.9	5.1	7.9	210	P	A	A	P
Above	CSU	2001	84.6	1.1	9.4	165	P	A	A	P
Akron	CSU	1994	96.6	4.5	9	165	P	A	A	P
Alliance	UNL	1993	104.8	10.6	7.8	165	P	P	A	P
Ankor	CSU	2002	95.4	8.3	7.9	210	P	A	A	A
Arapahoe	UNL	1988	100.3	2.4	8.1	210	P	A	A	P
Ardito	Italy	-	83.9	16.6	9.3	192	A	P	A	P
Argelato	Italy	-	62.4	22.2	9.6	192	A	P	A	P
Arkan	KSU-Manhattan	1982	92.1	20.5	8	210	A	P	A	A
Arlin	KSU-Manhattan	1992	72.4	2	9.1	165	P	A	A	A
Avalanche	CSU	2001	89.3	4.1	8.5	165,210	P	A	A	P
Avrora	Russia	-	89.9	18.4	9.8	192	A	P	A	P
Baca	CSU	1973	113.9	21.4	10.8	210	A	P	A	P
Bezostaya	Russia	-	82.8	18.1	8.9	192	A	P	A	P
Brevor	WSU	-	98.7	20.4	10	174	A	P	A	P
Brule	UNL	1981	99.2	2.5	7.5	165	P	A	A	P
Buckskin	UNL	1973	117.8	18.7	10.6	165,210	A	P	A	P
Cappelle Desprez	France	-	90.3	19.1	9.9	174	A	P	A	P
Centura	UNL	1983	112.5	20.3	7	192	A	P	A	P
Centurk	UNL	1971	107.1	17.3	9.8	192	A	P	A	P
Century	OSU	1986	88.1	2.5	9	165	P	A	A	P
Chisholm	OSU	1983	91.1	1.8	8	165	P	A	A	P
Ciano 67	Mexico	-	88.4	1.6	8.3	165	A	P	P	A
CO950043	CSU	2001	83.4	1.5	8.7	174	P	A	A	P
Colt	UNL	1983	85.2	2.1	8.7	210	P	A	A	P
Cossack	Westbred	1998	86.3	19.7	10.8	192	A	P	A	P
Cougar	UNL	1999	101.3	19.3	8.9	210	A	P	A	P

Table 1-1. Continued.

Entry	Origin†	Year	Plant height	GA sensitivity	Coleoptile length	Xgwm 261	*Rht-B1b*‡	*Rht-B1a*	*Rht-D1b*	*Rht-D1a*
			--------------------cm--------------------							
Custer	OSU	1994	86.5	4.3	8.5	165	P	A	A	P
Dodge	KSU-Manhattan	1986	89.5	1.4	7	210	P	A	A	P
Eagle	KSU-Hays	1970	105.7	21.3	9.5	210	A	P	A	P
Enhancer	Westbred	1998	85.2	4.6	9.5	165	P	A	A	P
Gaines	WSU	1961	75.8	2	8.7	174	A	P	P	A
Hail	CSU	1982	87.6	2	8.5	210	A	P	P	A
Halt	CSU	1994	83.2	2.6	6.8	165	P	A	A	P
Hawk	AgriPro	1980	72.4	3.2	10	192	A	P	P	P
Ike	KSU-Hays	1994	97.2	2.8	7.2	210	P	A	A	P
Jagalene	AgriPro	2001	83.6	7.9	8	210	P	A	A	P
Jagger	KSU-Manhattan	1994	85.3	5.9	7.9	210	P	A	A	P
Kalvesta	Westbred	1998	85.9	4.3	9.9	165	P	A	A	P
Kauz	Mexico	-	64.4	4	7.9	192	P	A	A	P
Kavkas	Russia	-	97.1	18.9	10.7	192	A	P	A	P
Lakin	KSU-Hays	2000	85.6	3.1	7.3	165	P	A	A	P
Lamar	CSU	1988	93.7	1.6	7.9	165	P	A	A	P
Lancota	UNL	1975	116.3	18	8.9	210	A	P	A	P
Laredo	AgriPro	1992	77.1	5.5	9.1	210	P	A	A	P
Larned	KSU-Hays	1988	110.6	25.8	11.7	210	A	P	A	P
Lindon	CSU	1975	65	21	9.5	192	A	P	A	P
Longhorn	AgriPro	1991	80.6	18.4	12.1	210	A	P	A	P
Lovrin 32	Romania	-	84.8	3.7	9.3	192	P	A	A	P
Mara	Italy	-	66.3	18.6	9.8	192	A	P	A	P
Mentana	Mexico	-	103.3	17.3	8.7	165	A	P	A	A
MTRWA116	MSU	-	95	12.4	8.5	174	A	P	A	P
Newton	KSU-Manhattan	1978	109.7	3.5	9.2	210	P	A	A	P
Norkan	KSU-Manhattan	1986	94.8	4.6	7.7	210	P	A	A	P
NuGaines	WSU	1965	77.5	0.9	9.1	174	P	A	A	P
Nuplains	UNL	1999	92.8	3.9	8.4	192	P	P	A	P
Ok101	OSU	2001	87.9	4	7.9	165	A	P	A	P
Ok102	OSU	2002	76.2	7.2	9.1	165	P	A	A	P
Opata 85	Mexico	-	74.8	2.7	7.2	165	P	A	A	P
Payne	OSU	1977	90.3	4.9	10.1	165	P	A	A	P

Table 1. Continued.

Entry	Origin†	Year	Plant height	GA sensitivity	Coleoptile length	Xgwm 261	*Rht-B1b*‡	*Rht-B1a*	*Rht-D1b*	*Rht-D1a*
				------------cm------------						
Platte	AgriPro	1995	69.7	4.1	7.7	210	P	A	A	P
Ponca	KSU-Manhattan	1951	126.6	23.0	10.3	210	A	P	A	P
Prairie Red	CSU	1998	84.8	3.4	9.2	165	P	A	A	P
Pronghorn	UNL	1995	107.1	18.7	9.9	192	A	P	A	P
Prowers 99	CSU	1999	104.7	24.8	12.1	165	A	P	A	P
Rawhide	UNL	1991	94.8	2.7	8.8	210	P	A	A	P
Rio Blanco	AgriPro	1988	66.8	4.5	9.0	192	P	A	A	P
Sage	KSU-Manhattan	1973	109.8	23.6	9.2	210	A	P	A	P
Saitama 27	Japan	-	82.4	0.8	8.0	165	P	A	A	P
Sandy	CSU	1981	105.4	19.4	10.8	192	A	P	A	A
Sava	Hungary	-	66.0	17.2	9.3	192	A	P	A	P
Scout 66	UNL	1967	117.9	22.3	8.9	210	A	P	A	P
Siouxland	UNL	1984	115.6	15.9	8.6	210	A	P	A	P
Stanton	KSU-Hays	2000	94.6	2.2	8.2	210	P	A	A	P
TAM 105	Texas-Amarillo	1979	91.9	2.4	7.8	165	P	A	A	P
TAM 107	Texas-Amarillo	1984	91.5	1.1	9.1	165	P	A	A	P
TAM 110	Texas-Amarillo	1996	78.9	4.5	7.9	165	P	A	A	A
TAM 111	Texas-Amarillo	2003	81.7	9.1	10.1	192	P	A	A	P
Thunderbird	AgriPro	1985	80.9	17.5	10.4	210	A	P	A	P
Thunderbolt	AgriPro	1999	91.6	8.0	8.5	165	P	A	A	P
Trego	KSU-Hays	1999	92.5	3.5	8.8	192	P	A	A	P
Vista	UNL	1992	89.6	1.3	7.5	210	P	A	A	P
Vona	CSU	1976	89.1	15.7	7.5	165	P	A	A	A
Wichita	KSU-Manhattan	1944	132.5	21.4	10.6	210	A	P	A	P
Yuma	CSU	1991	84.6	1.2	6.5	165	P	A	A	P
Yumar	CSU	1997	87.1	4.3	6.9	165	P	A	A	P
Mean			89.0	9.9	8.6					
LSD			8.3	12.6	2.4					
Range			62.4-132.5	2.7-25.8	6.5-12.1					

† CSU = Colorado State University; KSU = Kansas State University; MSU = Montana State University; OSU = Oklahoma State University; UNL = University of Nebraska at Lincoln; WSU = Washington State University.

‡ P = Presence of *Rht-B1b/Rht-B1a* or *Rht-D1b/Rht-D1a*; A = Absence of *Rht-B1b/Rht-B1a* or *Rht-D1b/Rht-D1a*.

allele. The differences between these results may be because of the 80 entries included in Bai et al. (2004), forty-five percent were experimental lines (Southern Regional Performance Trial) most of which will not be released as cultivars. In the current study, all the entries but one were released cultivars. Confirming previous research (Korzun et al., 1998; Worland et al., 1998) the 210 bp allele was not identified in the limited number of non-U.S. entries. Further validating Ahmad and Sorrells (2002), the 174 bp allele was found in just one Great Plains entry, CO950043.

Six different plant breeding programs in the region had released Great Plains entries found to carry the 192 bp allele. Although the 192 bp allele is found throughout the Great Plains region, it apparently has been utilized infrequently. Results from this study are consistent with Bai et al. (2004) who found the 192 bp allele in only 6% of the 80 HRW genotypes examined. Other alleles reported by Worland et al. (2001), Ahmad and Sorrells (2002), and Bai et al. (2004) were not identified among this collection.

Trait Associations between Xgwm 261 and *Rht* alleles

Differences ($P \leq 0.05$) were found within each Xgwm 261 allelic class for plant height, gibberellic acid sensitivity and coleoptile length (Table 1-1). The range of plant height within each allelic class varied from 23.7 cm within the 174 bp allelic class to 64.6 cm within the 210 bp allelic class. The wide range of plant height could be attributed to the mild plant growth conditions in the greenhouse along with different modifying genes in the genetic background of specific entries. The range of response for GA sensitivity and coleoptile length was similar within each of the Xgwm allelic classes suggesting few modifying genes for these traits.

Significant (P≤0.05) differences among the four Xgwm 261 allelic classes were observed for plant height, gibberellic acid sensitivity and coleoptile length (Table 1-2). Plant height of the 210 bp allelic class was greater (97.1 cm) than the other three allelic classes (87.9, 86.8, and 84.2 cm). The increase in plant height between the 192 bp and 174 bp classes (1.6 cm) and between 174 bp and 165 bp classes (1.1 cm) were not as great as reported earlier (Korzun et al., 1998; and Worland et al., 1998). The Xgwm 261 allelic classes differed in their GA sensitivity with the 165 bp and 174 bp allelic classes less sensitive to GA than the 192 bp and 210 bp allelic classes. Coleoptile length of the 192 bp allelic class was greater than the 165 bp class but not different from either the 174 bp or 210 bp classes. These observations differ from those of Bai et al. (2004) who reported no differences between the 192 bp and the 165 bp class for coleoptile elongation in hard winter wheat entries.

For each entry in our survey, the presence or absence of *Rht-B1b* and *Rht-D1b* alleles was identified through the use of PCR-based markers BF-MR1 and DF-MR2 (Ellis et al., 2002). Entries with both the *Rht-B1b* and *Rht-D1b* (dwarf phenotype) phenotype were not identified in this study. Differences (P≤0.05) between the *RhtB1b/D1b* (semi-dwarf) and *RhtB1a/D1a* (tall) allelic classes were observed for plant height, GA sensitivity and coleoptile length (Table 1-3). Entries with the *RhtB1b/D1b* genotypes exhibited shorter plant height (12%), less GA sensitivity (81%) and reduced coleoptile length (13%) compared with the *Rht-B1b/D1b* genotype. These observations corroborate earlier reports that *RhtB1b/D1b t* alleles are insensitive to GA resulting in shorter plants with shorter coleoptiles (Allan, 1989; Keyes and Paolillo, 1989).

Table 1-2. Least square means for plant height, gibberellic acid sensitivity, and coleoptile length among the Xgwm 261 allelic classes.

Xgwm 261 alleles	Plant Height	Gibberellic Acid Sensitivity	Coleoptile Length
	-------------------------cm	---------------------------------	
165 bp	87.9a†	5.0a	8.4a
174 bp	86.8a	9.4a	9.1ab
192 bp	84.2a	13.9b	9.4b
210 bp	97.1b	11.1b	8.9ab

† Values within a column followed by the same letter are not significantly different at the $\alpha = 0.05$ probability level according to the LSMEANS option of SAS PROC GLM.

Table 1-3. Least square means for plant height, gibberellic acid sensitivity and coleoptile length for *Rht-B1* and *Rht-D1* classes.

Semi-dwarf alleles	Plant Height	Gibberellic Acid Sensitivity	Coleoptile Length
	-----------------	cm	-----------------
RhtB1b or *Rht-D1b*	86.1a†	3.9a	8.3a
RhtB1a or *Rht-D1a*	97.3b	19.1b	9.7b

† Values within a column followed by the same letter are not significantly different at the $\alpha = 0.05$ probability level according to the LSMEANS option of SAS PROC GLM

Significant differences ($P \leq 0.05$) were observed when examining the trait response for alleles at the *Rht-B1* and *Rht-D1* loci within each Xgwm 261 allelic class (Table 1-4). Entries with the *Rht-B1a* + *Rht-D1a* alleles had increased plant height relative to the other combinations within the Xgwm 261-165 bp, 174 bp, and 210 bp allelic classes. GA sensitivity appeared to be suppressed in the presence of the *Rht-B1b* or *Rht-D1b* alleles in each Xgwm 261 allelic class. Differences for coleoptile length were observed in entries with the Xgwm 261-165 bp + *Rht-B1a/RhtD1a* and Xgwm 261-210 bp + *Rht-B1a/RhtD1a* alleles but not among the Xgwm 261-174 bp + *Rht-B1/RhtD1* or Xgwm 261-192 bp alleles. However, within the Xgwm 261 allelic classes, levels of significance and standard deviations of the *Rht-B1* and *Rht-D1* categories may have been influenced by population size (Table 1-4).

The primary objective of this survey was to characterize the Xgwm 261, *Rht-B1,* and *Rht-D1* alleles in each entry and assess the interaction between these alleles for plant height, GA sensitivity, and coleoptile length. Among the Xgwm 261-165 bp and 174 bp allelic classes, the *Rht-B1b* and *Rht-D1a* alleles were identified in 89% of the entries (Table 1) with corresponding semi-dwarf plant height, reduced GA sensitivity and short coleoptiles. However, 'Payne' carried the Xgwm 261-165 bp and *Rht-B1b* alleles but had long coleoptiles (10.1 cm). Furthermore 'Ok101' carried the Xgwm 261-165 bp and *Rht-B1a* alleles but had semi-dwarf plant height, decreased GA sensitivity, and short coleoptile length suggesting the Xgwm 261-165 bp allele suppressed *Rht-B1a/Rht-D1a* gene expression.

Eleven of the 20 entries in the Xgwm 261-192 bp allelic class were from the Great Plains. Five entries carried the *Rht-B1b* and *Rht-D1a* alleles, and had semi-dwarf

Table 1-4. Plant height, gibberellic acid sensitivity, and coleoptile length for Xgwm 261 and *Rht-B1* + *Rht-D1* allelic classes.

Xgwm 261 class	*Rht-B1* + *Rht-D1*	N	Plant Height	GA sensitivity	Coleoptile length
			-----------------	cm	-----------------
165 bp	*Rht-B1a* + *Rht-D1a*	3	98.6 ± 4.4 b†	15.4 ± 2.4 b	9.6 ± 0.6 b
	Rht-B1a + *Rht-D1b*	2	88.0 ± 7.7 a	2.7 ± 4.2 a	7.4 ± 1.1 a
	Rht-B1b + *Rht-D1a*	27	86.7 ± 1.5 a	3.9 ± 0.8 a	8.3 ± 0.2 a
174 bp	*Rht-B1a* + *Rht-D1a*	3	94.7 ± 2.4 b	17.3 ± 2.0 b	9.5 ± 0.4 a
	Rht-B1a + *Rht-D1b*	1	75.8 ± 4.2 a	2.0 ± 3.5 a	8.7 ± 0.7 a
	Rht-B1b + *Rht-D1a*	2	80.5 ± 3.0 a	1.2 ± 2.5 a	8.9 ± 0.5 a
192 bp	*Rht-B1a* + *Rht-D1a*	13	86.9 ± 4.6 a	18.9 ± 0.5 b	9.6 ± 0.3 a
	Rht-B1a + *Rht-D1b*	1	72.4 ± 16.7 a	3.2 ± 1.8 a	10.0 ± 0.9 a
	Rht-B1b + *Rht-D1a*	6	80.5 ± 6.8 a	4.8 ± 0.7 a	8.9 ± 0.4 a
210 bp	*Rht-B1a* + *Rht-D1a*	14	108.0 ± 3.8 b	20.6 ± 0.4 b	9.8 ± 0.7 b
	Rht-B1a + *Rht-D1b*	1	87.6 ± 13.6 a	2.0 ± 2.5 a	8.5 ± 1.0 a
	Rht-B1b + *Rht-D1a*	16	89.0 ± 3.5 a	4.0 ± 0.6 a	8.1 ± 0.3 a

† Values within a column followed by the same letter are not significantly different at the α = 0.05 probability level according to the LSMEANS option of SAS PROC GLM

plant height, decreased GA sensitivity, and short coleoptiles suggesting that in the entries the *Rht-B1b* suppressed the gene action of the Xgwm 261-192 bp allele or that the Xgwm 261-192 bp allele was not linked to *Rht8*. However, two ('Lindon' and 'Cossack') of the six entries carrying the Xgwm 261-192 bp and *Rht-B1a/Rht-D1a* alleles had semi-dwarf plant height, increased GA sensitivity, and longer coleoptiles indicating the Xgwm 261-192 bp allele suppressed the gene action of the *Rht-B1a* and *Rht-D1a* alleles possibly through linkage with *Rht8*.

Fifty five percent (17) of the Great Plains entries carried the Xgwm 261-210 bp and *Rht-B1b/Rht-D1b* allele and had semi-dwarf plant height, decreased GA sensitivity and short coleoptile length. Of the thirteen Great Plains entries that carried the Xgwm 261-210 bp and *Rht-B1a/Rht-D1a* alleles, ten entries had tall plant height, increased GA sensitivity, and long coleoptiles. However three entries ('Longhorn', 'Thunderbird', and 'Arkan') had semi-dwarf plant height along with increased GA sensitivity. Additionally, Longhorn and Thunderbird also had an increased coleoptile length. Longhorn and Thunderbird were initially suspected to carry the *Rht8* semi-dwarfing gene due to their semi-dwarf plant height, GA sensitivity, and longer coleoptiles. Additionally, the pedigree of these entries included a Yugoslavian line that could have carried *Rht8*, as many entries from this region have been shown to carry this gene (Worland et al., 1998). It is unclear whether Longhorn and Thunderbird show these phenotypes because they carry *Rht8* that is not linked with Xgwm 261-192 bp or they lack *Rht8* yet carry other unknown genes that confer their unique phenotypes.

CONCLUSIONS

In current and historical Great Plains entries, microsatellite marker Xgwm 261 identified four Xgwm 261 allelic classes of 165 bp, 174 bp, 192 bp, and 210 bp fragments. Differences for plant height, GA sensitivity and coleoptile length were observed among the Xgwm 261 allelic classes. Additionally, *Rht-B1* and *Rht-D1* alleles were identified for each entry and observations comparing the effects of *Rht* versus *rht* alleles revealed differences in plant height, GA sensitivity and coleoptile length. In previous research, semi-dwarf wheat populations with GA insensitivity (*Rht-B1b* and *Rht-D1b*) have also shown reduced coleoptile length. *Rht8* has been associated with semi-dwarf plant height, GA sensitivity and increased coleoptile length. However, there may not be any discernable advantage of utilizing *Rht8* for longer coleoptile length when other height inducing genes (*Rht-B1b, Rht-D1b*) are present because trait expression for GA sensitivity and increased coleoptile length are masked. In fact, no association was observed among Great Plains entries between plant height, GA sensitivity, and coleoptile length and any particular Xgwm 261 allele. In several entries, semi-dwarf plant height, GA sensitivity and long coleoptiles, as well as presence of the Xgwm 261-210 bp and *Rht-B1a/Rht-D1a* alleles, suggest that the linkage between *Rht8* and the Xgwm 261-192 bp allele may not be preserved in certain Great Plains genotypes. Therefore identifying entries with *Rht8* using the Xgwm 261-192 bp allele may not be possible in U.S. hard winter wheat germplasm.

CHAPTER TWO

Trait Associations at the Xgwm 261, *Rht-B1*, and *Rht-D1* Marker Loci in Two Winter Wheat Populations.

ABSTRACT

Higher grain yields led to the widespread adoption and deployment of the *Rht-B1b* and *Rht-D1b* semi-dwarfing genes in wheat (*Triticum aestivum* L.) cultivars. Reduced stand from shorter coleoptiles and yield instability when grown in low yielding environments have been cited as disadvantages. Interest in the gibberellic acid (GA) sensitive semi-dwarfing gene *Rht8* has increased due to the discovery of Xgwm261, a closely linked microsatellite marker, which may be useful to facilitate selection for *Rht8*. The objective of this study was to determine the effects of variation at the Xgwm 261, *Rht-B1*, and *Rht-D1* loci on GA sensitivity, coleoptile length, and agronomic traits of two winter wheat recombinant inbred line (RIL) populations. The two populations ['Longhorn'/'Akron' (LA), Longhorn/'Yuma' (LY)] were planted at five locations in eastern Colorado for two years and measurements for plant height, grain yield, test weight, heading date, above ground biomass, kernel weight and kernel number were recorded. Microsatellite markers Xgwm 261 (*Rht8*), BF-MR1 (*Rht-B1*), and DF-MR2 (*Rht-D1*) were used to characterize the RIL populations. Reduced GA sensitivity and coleoptile length, evaluated in greenhouse and growth room conditions, were observed in both LA and LY RILs carrying the *Rht-B1b* allele. Greater plant height (at all environments) and higher test weights (six of the eight environments) were observed in

LA RILS carrying the *Rht-B1a* alleles. Later heading date and greater plant height was consistently observed in LY RILs carrying the Xgwm 261-210 bp allele in combination with either *Rht-B1b* or *Rht-B1a.* Greater test weight and equivalent yields were observed in LY RILs carrying the Xgwm 261-210 bp + *Rht-B1a* alleles compared with the other allelic combinations. Observation of these agronomic traits, under the low production environments of the Great Plains, along with GA sensitivity and long coleoptile length suggests linkage of Xgwm 261-210 bp with *Rht8* in certain Great Plains wheat genotypes.

INTRODUCTION

The semi-dwarfing genes, *Rht-B1b* and *Rht-D1b* (previously known as *Rht1* and *Rht2*), have been used extensively in the development of semi-dwarf wheat cultivars worldwide. Higher grain yields led to widespread adoption of the *Rht* semi-dwarfing genes by CIMMYT and other public breeding programs facilitating the Green Revolution in wheat (Hedden, 2003). Semi-dwarf cultivars have higher grain yield potential through increased partitioning of assimilates to the head instead of the stem (Youssefian et al., 1992a, b). Additionally, plant height reduction improves straw strength under high input and irrigated conditions, resulting in reduced harvest losses from lodging (Hoogendoorn et al., 1990; Evans, 1993; Börner et al., 1997). Since the introduction of *Rht-B1b* and *Rht-D1b*, however, yield improvements have been inconsistent under high temperature and moisture stress conditions. Yield reductions are partially due to reduced stand establishment resulting from a shorter coleoptile (Allan, 1980; Gale and Youssefian, 1985) and spikelet fertility reductions when warm temperatures occur at the time of meiosis (Worland et al., 1998).

The Italian breeder Navareno Strampelli introduced *Rht8*, a different source of semi-dwarfism, into European wheats in the 1930's (Gale and Youssefian, 1985). *Rht8* may provide greater yield stability than *Rht-B1b* and *Rht-D1b* under low yielding environments (Worland et al., 1988b; Kertész et al., 1991). *Rht8* is currently used to reduce heights of commercial wheat cultivars grown in southern and Eastern Europe, China, Japan, and several Russian Federation countries (Worland and Law, 1986; Borojevic, 1998; Worland et al., 1998; Rebetzke and Richards, 2000).

Determination of *Rht* genotypes based on plant height alone is not possible. However, utilizing a gibberellic acid (GA) seedling screen, *Rht* genes can be separated into two groups: those that are sensitive to GA and those that are insensitive to GA (Worland et al., 1998). *Rht-B1b* and *Rht-D1b* alleles have been found to be GA insensitive thus allowing for their identification in experimental lines. GA insensitivity affects the plant pleiotropically, resulting in shorter coleoptiles, smaller seedling leaf areas, decreased internode length, and reduced plant height (Allan, 1989; Keyes and D.J. Paolillo, 1989; Beharev et al., 1998; Miralles et al., 1998). Shorter coleoptile length is associated with reduced seedling emergence and subsequent poor stand establishment unless sowing depth can be adequately reduced (Allan, 1980; Gale and Youssefian, 1985). Therefore, short coleoptile length is usually considered to be an undesirable characteristic in the low-precipitation regions of the Great Plains where deep planting is often necessary to place the seed into adequate soil moisture (Stockton et al., 1996).

Rht8 has been difficult to detect in segregating populations because it confers both a longer coleoptile and GA sensitivity similarly to *Rht-B1a* and *Rht-D1a* (tall) wheats (Worland, 1986; Korzun et al., 1998). Recently, this difficulty has been alleviated with the discovery of the microsatellite marker Xgwm 261 (Korzun et al., 1998). The Xgwm 261 marker was utilized by Korzun et al. (1998) in PCR analysis to identify three alleles distinguishable by fragment sizes of 165 bp, 174 bp, and 192 bp in two recombinant inbred line (RIL) populations developed from crosses of 'Mara' and 'Ciano 67' with 'Cappelle-Desprez'. A single recombinant individual from the Mara and Cappelle-Desprez RIL population was identified resulting in an estimated linkage of 0.6 cM between Xgwm 261 and *Rht8*. Korzun et al. (1998) concluded that Xgwm 261 may be useful as a diagnostic marker even though co-segregation was not absolute and the mapping populations were relatively small.

In the two RIL populations developed by Korzun et al. (1998), identification of the Xgwm 261 variants revealed lines with the Xgwm 261-192 bp allele to have 7 to 8 cm shorter plant height than lines with the Xgwm 261-174 bp allele. Additionally, lines with the Xgwm 261-174 bp allele had 3 cm shorter plant height than lines with the Xgwm 261-165 bp allele (Korzun et al., 1998).

Korzun et al. (1998) and Worland et al. (1998) evaluated numerous accessions from non-U.S. sources and found Xgwm 261 to be highly polymorphic with three main allelic variants: 165 bp, 174 bp, and 192 bp. Chebotar et al. (2001) performed additional screening with 27 accessions from the Ukrainian Research Institutes and found 88% to carry the Xgwm 261-192 bp allele. Ahmad and Sorrells (2002) screened 37 Soft U.S. wheats and four Hard Red Winter wheats and also identified the: 165 bp and 174 bp.

In the first study to evaluate hard winter wheat genotypes from the Great Plains region, Bai et al. (2004) examined 80 genotypes for allelic variants of Xgwm 261 and coleoptile length. The most common alleles of the hard winter wheat genotypes were the 165 bp, 174 bp, and 192 bp fragments, in agreement with Korzun et al. (1998), Worland et al. (1998), and Ahmad and Sorrells (2002). Other alleles identified among the Great Plains entries included the 195, 196, 197, 201, 203, 205, 210, and 215 bp fragments. Bai et al. (2004) concluded that the 192 bp allele was distributed in low frequencies among hard winter wheat breeding programs in the Great Plains. However, they were unable to detect an association between the 192 bp allele and coleoptile length and concluded that increased coleoptile length is not ensured by the presence of the 192 bp allele.

Additionally, they were not able to confirm the presence of *Rht8* among the U.S. accessions since the 192 bp allele is a marker linked to *Rht8* and not a part of the gene itself.

Plant height and coleoptile length are also affected by the *Rht-B1* and *Rht-D1* alleles but until recently, distinguishing between these alleles has not been possible. Ellis et al. (2002) developed PCR-based microsatellite markers that differentiate between the alleles at the *Rht-B1* and *Rht-D1* loci. Butler et al. (2005) utilized these microsatellite markers to identify alleles at the *Rht-B1* and *Rht-D1* loci in a RIL spring wheat population. Plant height was found to be relatively consistent across four environments when correlated with the identified allelic classes. Flintham et al. (1997) and Butler et al. (2005) both reported similar plant height reductions of 15.5% for *Rht-B1b* or *Rht-D1b*. However, Richards (1992) found greater plant height reductions (23%) for a single dwarfing gene emphasizing the importance of genetic background and environment in determining the extent of plant height reduction.

To date, no reports have documented the effect of allelic variation at GA sensitive and GA insensitive semi-dwarfing loci in the same segregating population. The objective of this study was therefore to determine the effects of allelic variation at the Xgwm 261, *Rht-B1*, and *Rht-D1* loci on GA sensitivity, coleoptile length, and agronomic traits of two winter wheat RIL populations.

MATERIAL AND METHODS

Population Development

Two populations of F4:6 (planted in 2004) and F4:7 (planted in 2005) RILs were developed by crossing 'Yuma' and 'Akron' with 'Longhorn'. Yuma (NS14/NS25/2/2*'Vona') and Akron ('TAM 107'/'Hail') are cultivars developed and released by Colorado State University with semi-dwarf height, gibberellic acid insensitivity, and short coleoptiles. Longhorn (NS2630-1/'Thunderbird'), a cultivar developed and released by AgriPro, was selected as the common parent because of its semi-dwarf height, gibberellic acid sensitivity, and long coleoptile. These characteristics, along with its pedigree (NS2630-1 originated from Yugoslavia where *Rht8* is common), suggested that Longhorn may be carrying *Rht8*. Marker screening using Xgwm 261 showed that Longhorn carried the 210 bp allele after the populations were developed. Seed harvested from F1 plants were advanced to the F4 generation by single seed descent.

In the 2002-2003 growing season, F4:F5 RILs from the Longhorn/Akron (LA) and Longhorn/Yuma (LY) populations were increased in Yuma, AZ. Seventy-five RILs from the LA population and sixty-five RILs from the LY population were randomly chosen for use in the field trials.

Field Trials

Field trials were conducted in 2003-2004 (2004) and 2004-2005 (2005) at five locations in eastern Colorado: Akron, Burlington, Julesburg, Walsh, and Fort Collins. All sites were non-irrigated except for Fort Collins where irrigation was applied with a linear overhead sprinkler system at approximately weekly intervals to achieve maximum yields.

The trials were designed as 15 x 10, α-0,1 rectangular lattices with two replicates (Patterson et al., 1978). Entries included RILs of both populations along with one occurrence of each parent, and 'Jagger' and 'Prowers 99' as check cultivars. All plots were planted 3.7 m long and six rows wide with, 25 cm spacing (except for Walsh at 30 cm) between rows (5.5 m^2 harvested area at all locations except Walsh at 6.03 m^2). In 2004, the Burlington site was lost due to a spring freeze event and the Walsh site was lost to a hail event on the eve of harvest. All sites were harvested in 2005. To facilitate presentation, environmental combinations (location and year) were coded as follows: Akron 2004, A04; Akron 2005, A05; Burlington 2005, B05; Fort Collins 2004, F04; Fort Collins 2005, F05; Julesburg 2004, J04; Julesburg 2005, J05; Walsh 2005, W05.

Data were recorded for days to heading, plant height, grain yield, test weight, above ground biomass, kernel number, and kernel weight. Utilizing the Julian calendar, days to heading was recorded as the day on which 50% of the spikes were fully visible above the flag leaf collar. These data were recorded at Julesburg and Fort Collins in 2004 and Akron and Fort Collins in 2005. Plant height was recorded at harvest and measured from the soil surface to the tip of the heads excluding the awns. Grain yield was determined with a small plot combine that harvested the entire plot. A sub-sample of grain from each plot was cleaned and used to determine test weight. At Akron and Burlington in 2005, test weight was not obtained for some plots due to low grain yield.

Above ground biomass samples were collected immediately before harvest at Akron and Fort Collins in 2004 and Julesburg and Fort Collins in 2005. A 1-m section of a single row was cut at the soil surface, placed in a large plastic bag, and weighed. Five heads from the biomass sample were randomly selected and threshed together. Kernel number and kernel weight were determined from this sample.

Molecular Marker Analysis

DNA was isolated from bulked leaves of five to six seedlings of each RIL according to the procedures of Riede and Anderson (1996). DNA was further purified with addition of 1 µL RNAase and then incubated at 37 °C for 30 min. Microsatellite marker Xgwm 261 was amplified in a 20 µL PCR reaction containing 80 ng DNA, 1x PCR buffer, 120 µM dNTP, 2.5 µM of the Xgwm 261 primer pairs and 0.8 units of *Taq* polymerase. The following touchdown thermal profile was used for DNA amplification: 3 min at 94 °C, 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C for five cycles, in which the annealing temperature was lowered by 1 °C per cycle; and 31 cycles in which the annealing temperature remained constant at 50 °C. Ten minutes at 72 °C was used for the final extension. Amplified DNA was loaded into 3% high resolution agarose gels (SFRA, Amresco, Solon, OH) and electrophoresed at 100 V for 3 h 30 min. Agarose gels were stained with ethidium bromide for 30 min, rinsed for 30 sec in deionized water, and then digitally photographed under ultraviolet light using the Alphaimager documentation system (Alpha Innotech Corp., San Leandro, CA). Molecular sizes of the DNA fragments were determined in relation to the Amplisize Molecular Ruler (BioRad, Richmond, CA) and comparison with three entries: Yuma (Xgwm 261-165 bp), Longhorn (Xgwm 261-210 bp), and Mara (Xgwm 261-192 bp).

Primer pairs developed by Ellis et al. (2002) were utilized to identify *Rht-B1* and *Rht-D1* alleles for the RILs. Identification of *Rht-Bl* alleles was based on the BF-MR1 primer pair and identification of *Rht-D1* alleles was based on the DF-MR2 primer pair.

The PCR conditions were the same as specified by Ellis et al. (2002) except that the annealing temperature for the BF-MR1 primer pair was increased to 62.4 °C. PCR products were loaded into 4% high resolution agarose gels and electrophoresed at 100 V for 3 h 30 min. Agarose gels were stained with ethidium bromide for 30 min, rinsed for 30 sec in deionized water, and then digitally photographed under ultraviolet light using the Alphaimager documentation system. Molecular sizes of the DNA fragments were determined in relation to the Amplisize Molecular Ruler (BioRad, Richmond, CA) and comparison with two entries: Yuma (*Rht-B1b*) and Longhorn (*Rht-B1a*).

Gibberellic Acid Sensitivity Measurement

Plastic 38.1 x 15.3 x 5.1 cm trays (Rubbermaid Co., Wooster, OH) were filled 3/4 full with coarse vermiculite (American Clay Works, Denver, CO). Trays were planted with ten test entries and two check entries (Mara and Yuma) in each tray. Ten seeds of each entry were planted in a single row within the tray and covered with a layer of vermiculite. Trays were placed in the greenhouse and watered, either with distilled water or a 25 ppm GA solution (Sigma-Aldrich Inc, St. Louis, MO) as necessary. After 21 d, plant height was measured from the seed to the tip of the tallest leaf. GA sensitivity was determined as the mean of the 10 seedlings treated with distilled water subtracted from the mean of the 10 seedlings treated with the GA solution. Experimental design was a randomized complete block with two replications. Replications were temporally blocked due to a two week separation between replications.

Coleoptile Length Measurement

Forty seeds, embryo side down, were placed approximately 1 cm apart and 2.2 cm from the bottom on pre-moistened 39.6 x 13.2 cm blotter paper (Anchor Paper Co., St. Paul, MN). A pre-moistened blotter paper was placed over the seeds and both sheets were loosely rolled and secured with a size no. 32 rubber band (S.P. Richards, Atlanta, GA). Samples were placed in a plastic 43.8 x 29.5 x 16.5 cm container (Rubber Maid Co., Wooster, OH) filled to a depth of 2 cm with distilled water. Containers were covered with foil and placed in a cold room at 4 °C for 4 d. Samples were then placed in a Percival incubator (Percival Scientific, Inc., Boone, IA) at 16 °C for 16 d. Measurements were taken from the seed to the top of the coleoptile. Experimental design was a randomized complete block with two replications. Replications were temporally blocked due to a one month separation between replications.

Data Analysis

Estimated means for plant height, grain yield, test weight, heading date, above ground biomass, kernel number, and kernel weight were determined utilizing an analysis of variance with SAS PROC Mixed (SAS Institute Inc., 1999), in which the model adjusted for incomplete block effects based on the rectangular lattice design (Patterson et al., 1978). Incomplete blocks were considered to be random effects while replications and RILs were considered as fixed effects. The magnitude of the effects of alleles for the Xgwm 261 and *Rht-B1* loci on measured variables was determined utilizing SAS PROC GLM (SAS Institute Inc., 1999). Loci were included as main effects; interaction was done to test for epistasis. Frequency distributions of least square means for GA sensitivity and coleoptile length were plotted. The Shapiro-Wilk test for normality was conducted with the SAS UNIVARIATE procedure was to evaluate normality of trait distribution (SAS Institute Inc., 1999). A chi-square analysis was performed to detect significant deviation from expected Mendelian segregation ratios for the two markers. Expected ratios for were 1:1 at the individual Xgwm261 and *Rht-B1* loci and 1:1:1:1 for the allelic combination of the four genotypic classes.

RESULTS AND DISCUSSION

Trial Conditions

Planting conditions at Akron, Burlington, Julesburg and Walsh in fall of 2003 were poor due to dry soil conditions. Adequate stands were obtained at these locations except for Akron where soil compaction led to strips of missing plots across several ranges. At Akron gap notes were taken and plot size was adjusted accordingly. Dry conditions continued through the winter and spring except for timely rains in April. Moderate temperatures and frequent rains began in mid-June and continued into July, providing mild grain filling conditions and leading to serious weed pressure at Julesburg resulting from incomplete canopy cover due to the earlier drought stress. Fort Collins, an irrigated site, had adequate soil moisture throughout the growing season and also benefited from mild temperatures during grain filling. Only the yield trials at Akron, Fort Collins, and Julesburg were harvested. At Burlington, temperatures dropped to -2.2 °C for five hours on May 13, 2004 when the wheat was at the early boot to late boot stage. The cold temperatures resulted in high levels of spikelet sterility throughout the yield trials and the trial was abandoned. The trial at Walsh sustained a damaging hail event June 15, 2004 and was also abandoned.

In 2005, the trials at each location were planted with good topsoil moisture which was sufficient for abundant plant growth through the winter months into April. In eastern Colorado, from late April into May, there was a period of drought stress punctuated by high temperatures that adversely affected trials at Akron, Burlington, and Julesburg. At Akron and Burlington, the combination of drought and heat stress at heading caused sterility in the emerged spikelet and in some cases the plants ceased growing, greatly reducing plant height, grain yield, and test weight. Test weight at Akron and Burlington in 2005 was severely impacted and was not included in the analysis. Julesburg was slightly later in heading than Akron and Burlington, and thus was not heading during the peak heat stress. Plant height was reduced at Julesburg but grain yield, kernel weight, kernel number, test weight, and above ground biomass were not severely affected. At Walsh, excellent moisture and moderate temperatures from late May to early June promoted severe stripe rust infection. However, segregation among the RILs was not observed and thus stripe rust did not differentially affect the measured variables. Fort Collins, an irrigated site, had adequate soil moisture throughout the growing season but yield was reduced compared with 2004 due to higher temperatures during grain filling and a severe stripe rust infection. All yield trials were harvested in 2005.

Plant height, grain yield, and test weight for the LA and LY RIL populations differed ($P \leq 0.05$) among the eight environments (Tables 2-1 and 2-2). At Akron, drought and heat stress in 2005 compared with 2004 resulted in shorter plant height and lower grain yield. The drought and heat stress at Burlington in 2005 resulted in reduced plant height and grain yield compared with the other dryland locations with only Akron (2005) reporting lower values for these traits. In 2005, Fort Collins, an irrigated site, did not experience the extreme heat and drought stress of the other eastern Colorado sites. Due to higher temperatures during the spring and early summer compared with 2004, however, plant height at Fort Collins in 2005 was shorter and both grain yield and test weight were lower for the LA RILs. Julesburg experienced low levels of soil moisture from planting until early June in 2004, when rain accompanied by mild temperatures occurred during

Table 2-1. Plant height, grain yield, and test weight means for the Longhorn/Akron recombinant inbred line population grown in eight environments.

Environment Abbreviation	Irrigation	Plant Height Mean	Plant Height Range	Grain Yield Mean	Grain Yield Range	Test Weight Mean	Test Weight Range
		--------cm----------		---------kg ha^{-1}---------		------------kg m^{-3}---------	
A04	Dryland	57.0c†	44-68	2782f	1437-3629	760c	721-786
A05	Dryland	42.2a	33-51	656a	21-1486	-	-
B05	Dryland	47.5b	40-55	1588b	387-2921	-	-
F04	Irrigated	68.6e	53-84	5137h	3538-6216	786d	749-821
F05	Irrigated	62.5d	48-77	3445g	1637-4897	783d	745-816
J04	Dryland	43.1a	33-55	1856c	1289-2549	744b	695-782
J05	Dryland	56.3c	48-66	2507e	1386-3437	699a	689-729
W05	Dryland	67.5e	51-81	2235d	938-3633	748b	727-807

† Within a column, means followed by the same letter do not differ significantly at $\alpha = 0.05$ according to the LSMEANS option of SAS PROC GLM.

Table 2-2. Plant height, grain yield, and test weight means for the Longhorn/Yuma recombinant inbred line population grown in eight environments.

Environmental Abbreviations	Irrigation	Plant Height Mean	Plant Height Range	Grain Yield Mean	Grain Yield Range	Test Weight Mean	Test Weight Range
		--------------cm-----------		-----------kg ha^{-1}---------		---------- kg m^{-3}-----------	
A04	Dryland	54.1e†	42-64	2675d	1129-3787	756b	745-791
A05	Dryland	39.8a	31-46	600a	21-1290	-	-
B05	Dryland	43.3b	37-51	961b	92-2252	-	-
F04	Irrigated	66.4h	53-79	4392f	3219-5570	791cd	755-816
F05	Irrigated	59.6f	48-68	3175e	1129-4330	801cd	781-836
J04	Dryland	46.0c	40-57	1999c	1115-2631	760b	706-794
J05	Dryland	54.3e	46-62	2549d	1781-3408	727a	702-768
W05	Dryland	70.4g	55-86	2987e	1844-4076	781bc	750-817

† Within a column, means followed by the same letter do not differ significantly at $\alpha = 0.05$ according to the LSMEANS option of SAS PROC GLM.

grain filling resulting in reduced plant height and lower grain yield, but higher test weight. In 2005, Walsh had the greatest plant height but the stripe rust infection resulted in similar grain yield and test weight compared with the other Colorado locations.

Longhorn/Akron RIL Population

Molecular marker analysis found Longhorn to have the Xgwm 261-210 bp, *Rht-B1a*, and *Rht-D1a* alleles. Akron was heterogeneous for alleles at the Xgwm 261 locus as some plants carried the 165 bp allele and some carried the 210 bp allele. The Akron plant that was used to generate the cross must have carried the 210 bp allele and all RILs thus carried this fragment. As a consequence, the effects at this locus could not be evaluated. Similarly no segregation was observed among the RILs for alleles at the Rht-D1 locus as both parents carried the Rht-D1b allele. Longhorn (*Rht-B1a*/*Rht-D1a*) and Akron (*Rht-B1b*/*Rht-D1a*) differed at the *Rht-B1* locus thus enabling evaluation of allelic effects of this locus. The *Rht-B1b* allele was found in 36 of the RILs while the *Rht-B1a* allele was found in 39 of the RILs. Ratios of the two allelic classes were examined with a Chi-square analysis and there was agreement with the 1:1 expectation ($P \leq 0.05$).

Among the RILs, differences ($P \leq 0.05$) were observed between the *Rht-B1b* and *Rht-B1a* marker genotypes for GA sensitivity (Table 2-3). Frequency distributions for the RILs containing the *Rht-B1b* and *Rht-B1a* allele showed the distributions to be non-normal for GA sensitivity. RILs appeared to segregate according to the *Rht-B1* allele they were carrying from the parent cultivars Akron (2.0 cm) and Longhorn (19.0 cm). Jagger, Akron, Yuma, and the RILs carrying the *Rht-B1b* allele were shorter than Prowers 99, Longhorn, and the RILs carrying the *Rht-B1a* allele. RILs carrying the *Rht-B1b* allele were less sensitivity to GA than RILs carrying the *Rht-B1a* allele.

Table 2-3. Mean values of the Xgwm 261, *Rht-B1*, and combined allelic classes for coleoptile length and gibberellic sensitivity in the Longhorn/Akron, Longhorn/Yuma recombinant inbred lines, and check cultivars.

Genotype	Xgwm 261 and *Rht-B1* alleles	GA sensitivity	Coleoptile length
		-------------------cm	-------------------
LA RILs†	Xgwm 261-210 + *Rht-B1b*	3.9a‡	9.3a
	Xgwm 261-210 + *Rht-B1a*	15.8b	10.1b
LY RILs	Xgwm 261-165 bp	9.6a	8.8a
	Xgwm 261-210 bp	9.8a	9.1a
	Rht-B1b	4.3a	8.2a
	Rht-B1a	15.2b	9.6b
	Xgwm 261-165 + *Rht-B1b*	4.3a	8.2a
	Xgwm 261-165 + *Rht-B1a*	15.0b	9.3b
	Xgwm 261-210 + *Rht-B1b*	4.3a	8.2a
	Xgwm 261-210 + *Rht-B1a*	15.3b	10.0b
Jagger	Xgwm 261-210 + *Rht-B1b*	4.0a§	7.4b
Prowers 99	Xgwm 261-165 + *Rht-B1a*	17.2b	9.3d
Akron	Xgwm 261-210 + *Rht-B1b*	2.2a	8.5c
Yuma	Xgwm 261-165 + *Rht-B1b*	4.3a	6.7a
Longhorn	Xgwm 261-210 + *Rht-B1a*	20.4c	9.0d

† LA = Longhorn/Akron; LY = Longhorn/Yuma.

‡ For the recombinant inbred lines, means followed by the same letter do not differ significantly at $\alpha = 0.05$ according to the LSMEANS option of SAS PROC GLM.

§ For the cultivars, means followed by the same letter do not differ significantly at $\alpha = 0.05$ according to the LSMEANS option of SAS PROC GLM.

In contrast to GA sensitivity, RILs exhibited a normal frequency distribution for coleoptile length ($P \leq 0.05$). RILs and check cultivars carrying the *Rht-B1b* allele had shorter ($P \leq 0.05$) coleoptile length (Table 2-3) than RILs and check cultivars carrying the *Rht-B1a* allele. RILs containing the *Rht-B1b* allele had coleoptile length of 9.3 cm while lines with the *Rht-B1a* allele were 10.1 cm.

RILs carrying the *Rht-B1b* allele were shorter ($P \leq 0.05$) compared with RILs carrying the *Rht-B1a* allele in each of the eight environments (Table 2-4), with an average plant height reduction across the dryland and irrigated environments of 10.4%. Differences in plant height between the two allelic classes ranged from 1.5 cm at B05 to 4.0 cm at F04. Furthermore, among the check cultivars, Prowers 99 (Xgwm 261-165 bp and *Rht-B1a*) was taller than Jagger, Yuma, and Akron carrying the165 bp or 210 allele and *Rht-B1b* as well as Longhorn (Xgwm 261-210 bp and *Rht-B1a*) at Akron and Fort Collins in 2004 and Akron, Fort Collins, and Walsh in 2005 (Table 2-5).

In this study, the plant height reduction (10.2%) observed between the *Rht-B1b* and *Rht-B1a* allelic classes was lower than reported by Butler et al. (2005), Flintham et al. (1997), and Richards et al. (1992). Using a spring wheat RIL population grown under two moisture regimes, Butler et al. (2005) reported an average plant height reduction of 14.8% attributed to the *Rht-B1b* allele. Utilizing near-isogenic winter wheat lines, Flintham et al. (1997) found the *Rht-B1b* allele to account for a plant height reduction of 15.5% while Richards et al. (1992) found plant height to be reduced 23% for near-isogenic spring wheat lines containing the *Rht-B1b* allele. During the two years of this study, phenotypic expression for plant height at the dryland environments was inhibited due to the harsh environmental conditions. At the irrigated environments, expression of

Table 2-4. Mean values of the *Rht-B1b* and *Rht-B1a* allelic classes for traits measured of the Longhorn/Akron recombinant inbred lines in eight environments.

		Environment							
Trait (units)	Locus	A04†	A05	B05	F04	F05	J04	J05	W05
Plant height (cm)	*Rht-B1b*	53.1a‡	39.8a	45.5a	64.0a	59.8a	40.9a	52.7a	63.3a
	Rht-B1a	60.3b	44.7b	48.8b	72.7b	65.5b	45.1b	58.8b	71.3b
Grain yield (kg ha^{-1})	*Rht-B1b*	2712a	514a	1456a	5230a	3438a	1801a	2460a	2198a
	Rht-B1a	2849a	791b	1713b	5048a	3453a	1905a	2553a	2271a
Test weight (kg m^{-3})	*Rht-B1b*	757a	-	-	781a	778a	739a	685a	726a
	Rht-B1a	763b	-	-	793b	789b	749b	705b	755a
Kernel weight (mg kernel^{-1})	*Rht-B1b*	28.4a	-	-	30.9a	28.1a	-	25.5b	-
	Rht-B1a	29.8b	-	-	33.5b	29.8a	-	24.4a	-
Kernel number (kernels head^{-1})	*Rht-B1b*	26.2a	-	-	33.6b	22.2a	-	18.6a	-
	Rht-B1a	26.1a	-	-	31.1a	25.1a	-	19.9a	-
Heading date (Julian calendar)	*Rht-B1b*	-	142a	-	143a	145a	145a	-	-
	Rht-B1a	-	142a	-	143a	144a	145a	-	-
Above ground biomass (g m^{-1})	*Rht-B1b*	290a	-	-	451a	400a	-	385a	-
	Rht-B1a	327b	-	-	477a	399a	-	398b	-

† Environment abbreviations are as follows: A04, Akron 2004; A05, Akron 2005; B05, Burlington 2005; F04, Fort Collins 2004; F05, Fort Collins 2005, J04, Julesburg 2004; J05, Julesburg 2005; W05, Walsh 2005.

‡ For each trait within a column, means followed by the same letter do not differ significantly at α = 0.05 according to the LSMEANS option of SAS PROC GLM.

Table 2-5. Mean values of Jagger, Prowers 99, Akron, Yuma, and Longhorn for traits measured in eight environments.

			Environment							
Trait (units)	Entries	Alleles†	A04‡	A05	B05	F04	F05	J04	J05	W05
Plant height (cm)	Jagger	210 + *Rht-B1b*	56.5a§	44.0bc	48.4a	69.3a	59.4a	44.4a	58.3a	71.9a
	Prowers 99	165 + *Rht-B1a*	64.9b	45.3c	49.5a	78.1b	67.5b	49.5a	61.8a	79.9b
	Akron	210 + *Rht-B1b*	59.4ab	36.7ab	42.0a	63.8a	61.6a	43.8a	56.8a	68.2a
	Yuma	165 + *Rht-B1b*	55.0a	34.1a	43.3a	66.0a	60.3a	38.5a	56.5a	69.1a
	Longhorn	210 + *Rht-B1a*	57.2a	45.1c	46.6a	71.5a	56.5a	46.0a	58.7a	69.3a
Grain yield (kg ha^{-1})	Jagger	210 + *Rht-B1b*	2315a	712ab	2523a	5316a	2930a	2132a	2559a	3516b
	Prowers 99	165 + *Rht-B1a*	3004a	1143b	1835a	5458a	3918a	2262a	2754a	3682b
	Akron	210 + *Rht-B1b*	2750a	696ab	1320a	4441a	3868a	1910a	2888a	2983ab
	Yuma	165 + *Rht-B1b*	2558a	547a	1942a	5563a	3515a	2156a	3098a	3611b
	Longhorn	210 + *Rht-B1a*	2880a	792ab	1635a	4598a	3195a	1837a	2876a	2264a
Test weight (kg m^{-3})	Jagger	210 + *Rht-B1b*	762ab	-	688a	774a	776a	730a	536a	781a
	Prowers 99	165 + *Rht-B1a*	775b	-	750a	819c	819c	779d	713a	804b
	Akron	210 + *Rht-B1b*	774ab	-	-	778a	784ab	743b	703a	753a
	Yuma	165 + *Rht-B1b*	742a	-	751a	785b	805bc	737ab	716a	774a
	Longhorn	210 + *Rht-B1a*	768ab	-	741a	795b	800bc	764c	729a	785a
Kernel weight (mg kernel^{-1})	Jagger	210 + *Rht-B1b*	29.6ab	-	-	30.2a	30.7a	-	27.5a	-
	Prowers 99	165 + *Rht-B1a*	31.6b	-	-	36.2a	34.6a	-	25.3a	-
	Akron	210 + *Rht-B1b*	28.9ab	-	-	30.5a	32.1a	-	27.6a	-
	Yuma	165 + *Rht-B1b*	27.2a	-	-	30.9a	28.8a	-	24.3a	-
	Longhorn	210 + *Rht-B1a*	28.7a	-	-	39.3a	34.3a	-	25.9a	-
Kernel number (kernels head^{-1})	Jagger	210 + *Rht-B1b*	21.3a	-	-	34.5ab	21.5a	-	22.6a	-
	Prowers 99	165 + *Rht-B1a*	28.0b	-	-	29.3a	16.7a	-	20.8a	-
	Akron	210 + *Rht-B1b*	23.1ab	-	-	30.7ab	20.0a	-	17.9a	-
	Yuma	165 + *Rht-B1b*	28.0b	-	-	39.9b	23.2a	-	23.5a	-
	Longhorn	210 + *Rht-B1a*	24.4ab	-	-	30.6a	19.9a	-	20.2a	-

Table 2-5. continued.

			Environment							
Trait (units)	Entries	Alleles†	A04‡	A05	B05	F04	F05	J04	J05	W05
Heading date (Julian calendar)	Jagger	210 + *Rht-B1b*	-	140.5a§	-	140.3a	141.4a	137.5a	-	-
	Prowers 99	165 + *Rht-B1a*	-	144.1c	-	144.5c	145.7c	145.6b	-	-
	Akron	210 + *Rht-B1b*	-	142.4bc	-	142.1b	145.2b	142.5b	-	-
	Yuma	165 + *Rht-B1b*	-	143.3c	-	142.3b	144.8c	143.2b	-	-
	Longhorn	210 + *Rht-B1a*	-	141.5ab	-	143.3bc	141.7a	144.7b	-	-
Above ground biomass (g m^{-1})	Jagger	210 + *Rht-B1b*	311b	-	-	360ab	374a	-	463b	-
	Prowers 99	165 + *Rht-B1a*	313b	-	-	515bc	397a	-	359a	-
	Akron	210 + *Rht-B1b*	265ab	-	-	388b	373a	-	343a	-
	Yuma	165 + *Rht-B1b*	247a	-	-	529c	412a	-	422ab	-
	Longhorn	210 + *Rht-B1a*	292ab	-	-	438abc	432a	-	399ab	-

† Abbreviations for Xgwm 261 alleles are as follows: Xgwm 261-165 bp, 165; Xgwm 261-210 bp, 210.

‡ Environment abbreviations are as follows: A04, Akron 2004; A05, Akron 2005; B05, Burlington 2005; F04, Fort Collins 2004; F05, Fort Collins 2005, J04, Julesburg 2004; J05, Julesburg 2005; W05, Walsh 2005.

§ For each trait within a column, means followed by the same letter do not differ significantly at α = 0.05 according to the LSMEANS option of SAS PROC GLM.

plant height was greater due to the well-watered growing conditions although the reduction in plant height (10.2%) between the *Rht-B1b* and *Rht-B1a* allelic classes was comparable with the dryland environments (10.1%). Butler et al. (2005) also reported that plant height expression was relatively consistent across a broad range of environments. In this study, the *Rht-B1b* allele accounted for 34% (across eight environments) of the phenotypic variance for plant height whereas Butler et al. (2005) found a greater amount of the phenotypic variance (59% for the four environments) explained by the *Rht-B1b* allele. The differences between Butler et al. (2005) and this study for observed phenotypic variance accounted for by the *Rht-B1b* allele are likely due to differences in genetic background of the RIL populations studied, environmental conditions, and their interaction.

Grain yield was comparable between the *Rht-B1b* and *Rht-B1a* allelic classes at all environments except for Akron and Burlington in 2005 (Table 2-4). At Akron and Burlington (2005), with severe drought and heat stress at heading, RILs carrying the *Rht-B1a* allele had higher yield than RILs with the *Rht-B1b* allele. These results corroborate earlier reports of the association of the *Rht-B1b* allele with lower grain yields in environments with increased drought or temperature stress (Kertesz et al., 1991; Richards, 1992a). At Akron and Walsh (2005), Prowers 99 carrying the Xgwm 261-165 bp and *Rht-B1a* alleles had the highest grain yield of the check cultivars (Table 2-5).

While yield differences between the allelic classes were similar, the RILs carrying the *Rht-B1a* allele had increased test weight compared with the RILs carrying the *Rht-B1b* allele at Akron, Fort Collins, and Julesburg in 2004 and Fort Collins and Julesburg in 2005 (Table 2-4). Prowers and Longhorn, cultivars carrying the *Rht-B1a* allele also showed higher test weights compared with Jagger, Akron, and Yuma which carry the *Rht-B1b* allele (Table 2-5). These results were consistant with Butler et al. (2005) who reported that spring wheat lines with the *Rht-B1a* allele had higher test weight at each of four environments. Additionally, Guttieri et al. (2001) evaluated 16 spring wheat cultivars and observed test weights of tall cultivars to be greater than that of semi-dwarf cultivars. The results of this study along with those of Butler et al. (2005) and Guttieri et al. (2001) suggest that RILs with the *Rht-B1a* allele may be better able to fill the grain under increased heat and drought stress.

Differences ($P \leq 0.05$) in kernel weight were observed between the RILs carrying the *Rht-B1b* allele and RILs carrying the *Rht-B1a* allele at Akron and Fort Collins (2004) where RILs carrying the *Rht-B1a* allele had greater kernel weight than RILs carrying the *Rht-B1b* allele (Table 2-4). These results were comparable with results reported by Butler et al. (2005) and Kertész et al. (1991) in which entries with the *Rht-B1a* allele had greater kernel weight than entries carrying the *Rht-B1b* allele. Higher kernel weight was also observed in Prowers 99 (*Rht-B1a*) at Akron (2004) (Table 2-5). RILs with the *Rht-B1b* allele had higher kernel number under the well-watered environment of F04 than RILs with the *Rht-B1a* allele ($P \leq 0.05$) (Table 2-4). However, Flintham et al. (1997) observed greater kernel number among the lines with the *Rht-B1b* allele compared with lines with the *Rht-B1a* allele at dryland and irrigated environments.

No differences in heading date between RILs with the *Rht-B1b* and *Rht-B1a* allele were observed at any location (Table 2-4). RILs with the *Rht-B1a* allele at Akron (2004) had greater ($P \leq 0.05$) above ground biomass compared with RILs with the *Rht-B1b* allele (Table 2-4). However, no differences in above ground biomass were observed between allelic classes at the irrigated environments. Additionally, the cultivars showed no consistent trends between environments or allelic classes for above ground biomass (Table 2-5). These results corroborate reports by Butler et al. (2004), who observed greater above ground biomass in spring wheat lines with the *Rht-B1a* allele than in lines with *Rht-B1b* allele at three of four locations studied.

Longhorn/Yuma RIL Population

Molecular marker analysis found Longhorn to carry the Xgwm 261-210 bp, *Rht-B1a*, and *Rht-D1a* alleles while Yuma carried the Xgwm 261-165 bp, *Rht-B1b*, and *Rht-D1a* alleles. Because the *Rht-D1a* allele was found in both parental lines it was not included in the remaining analysis. Twenty-eight LY RIL lines carried the Xgwm 261-165 bp allele, thirty-five carried the Xgwm 261-210 bp allele and seven were heterozygous for the two marker fragments. The seven heterozygous lines were eliminated from the analysis; ratios of the remaining were examined with Chi-square and were in agreement with the 1:1 expectation ($P \leq 0.05$). The *Rht-B1b* allele was found in 38 of the LY RILs while the *Rht-B1a* allele was found in 27 RILs. Ratios of the two allelic classes were examined with Chi-square and there was agreement with the 1:1 expectation ($P \leq 0.05$). The four allelic classes Xgwm 261-165 + *Rht-B1b* (18 RILs), Xgwm 261-165 + *Rht-B1a* (10 RILs), Xgwm 261-210 + *Rht-B1b* (22 RILs), and Xgwm 261-210 + *Rht-B1a* (13 RILs) were examined with Chi-square analysis and found to be in agreement with an expected 1:1:1:1 ratio ($P \leq 0.05$).

Among the RILs, differences were found between the *Rht-B1b* and *Rht-B1a* allele classes (Table 2-3) for GA sensitivity. Frequency distributions for the LY RILs containing the *Rht-B1b* and *Rht-B1a* allele were not normal. No differences were observed between RILs carrying the Xgwm 261-165bp allele and the RILs carrying the Xgwm261-210bp allele (Table 2-3) suggesting that the Xgwm261 locus did not affect GA sensitivity. However, RILs carrying the *Rht-B1b* allele (4.3 cm) were significantly ($P \leq 0.05$) less sensitive to GA than RILs with the *Rht-B1a* allele (15.2 cm)

In contrast to GA sensitivity, RILs exhibited a normal frequency distribution ($P \leq 0.05$) for coleoptile length (Table 2-3). No differences were observed between RILs carrying the Xgwm-165bp allele (8.8 cm) and RILs carrying the Xgwm261-210bp allele (9.1 cm). However, differences ($P \leq 0.05$) were observed between the RILs carrying the *Rht-B1b* allele (8.2 cm) compared with the RILs carrying the *Rht-B1a* allele (9.6 cm).

The RILs carrying the Xgwm 261-165 bp allele had an average plant height reduction of 4% across the dryland and irrigated environments compared with RILs carrying the Xgwm 261-210 bp allele. Differences in plant height ranged from 0.1 cm at Akron and Burlington in 2005 to 2.0 cm at Fort Collins in 2005 (Table 2-6). However, the RILs carrying the *Rht-B1b* allele showed an average plant height reduction of 9% across the six dryland environments compared with RILs carrying the *Rht-B1a* allele, similar to the 10.2% height reduction observed in the LA RIL population. However, the *Rht-B1b* allelic class showed an average plant height reduction of 7% compared with the *Rht-B1a* allelic class across the two irrigated environments. Of the check cultivars, Jagger, Akron and Yuma (*Rht-B1b*) were shorter than Prowers 99 and Longhorn (*Rht-B1a*) (Table 2-5). Among the RILs, the *Rht-B1b* allele accounted for 23% (across eight environments) of the phenotypic variance for plant height which was less than that observed in the LA RIL population (34%). In contrast, Butler et al. (2005) found a greater amount of the phenotypic variance (59% for the four environments) explained by the

Table 2-6. Mean values of the Xgmw261, *Rht-B1,* and combined allelic classes for traits measured of the Longhorn/Yuma recombinant inbred lines in eight environments.

		Environment							
Trait	Allele	A04†	A05	B05	F04	F05	J04	J05	W05
Plant Height (cm)	Xgwm 261-165	52.2a‡	40.0a	43.4a	65.4a	57.3a	44.6a	52.5a	69.7a
	Xgwm 261-210	56.1b	40.3a	43.6a	68.3b	61.8b	47.9b	55.6b	71.8a
	Rht-B1b	51.7a	38.6a	42.1a	63.3a	58.5a	44.2a	52.1a	67.0a
	Rht-B1a	56.6b	41.7b	44.8b	70.3b	60.6a	48.4b	56.1b	74.5b
	Xgwm 261-165 + *Rht-B1b*	50.8a	38.4a	42.6ab	63.0a	57.0a	43.2a	50.8a	67.4a
	Xgwm 261-165 + *Rht-B1a*	53.7a	41.6b	44.2ab	67.7b	57.6a	46.0a	54.3b	72.1b
	Xgwm 261-210 + *Rht-B1b*	52.7a	38.8a	41.6a	63.6a	59.9b	45.1a	53.3b	66.6a
	Xgwm 261-210 + *Rht-B1a*	59.5b	41.8b	45.5bc	73.0c	63.6c	50.8b	58.0c	77.0b
Grain Yield (kg ha^{-1})	Xgwm 261-165	2566a	553a	959a	4380a	2986a	1899a	2495a	2903a
	Xgwm 261-210	2667a	606a	918a	4314a	3249a	2043a	2599a	2960a
	Rht-B1b	2734a	605a	954a	4529b	3236a	1917a	2473a	3040a
	Rht-B1a	2499a	554a	923a	4165a	3000a	2025a	2621a	2822a
	Xgwm 261-165 + *Rht-B1b*	2710a	611a	935a	4512c	3164a	1851a	2437a	3079a
	Xgwm 261-165 + *Rht-B1a*	2423a	495a	984 a	4248b	2808a	1946a	2554a	2726a
	Xgwm 261-210 + *Rht-B1b*	2759a	598a	974a	4547c	3307a	1983a	2509a	3000a
	Xgwm 261-210 + *Rht-B1a*	2574a	613a	862a	4082ab	3191a	2104a	2690a	2918a
Test Weight (kg m^{-3})	Xgwm 261-165	732a	-	-	794a	806a	764a	734b	783a
	Xgwm 261-210	766a	-	-	791a	798a	762a	722a	782a
	Rht-B1b	764a	-	-	789a	797a	755a	726a	778a
	Rht-B1a	734a	-	-	796b	808a	770b	731a	787b
	Xgwm 261-165 + *Rht-B1b*	763ab	-	-	789a	806a	755a	732b	782ab
	Xgwm 261-165 + *Rht-B1a*	701a	-	-	798b	806a	773b	737b	785b
	Xgwm 261-210 + *Rht-B1b*	765b	-	-	789a	797a	755a	721a	775a
	Xgwm 261-210 + *Rht-B1a*	766b	-	-	794a	810a	768b	724ab	789b

Table 2-6. continued.

		Environment							
Trait	Allele	A04†	A05	B05	F04	F05	J04	J05	W05
Kernel weight (mg kernel^{-1})	Xgwm 261-165	27.4a‡	-	-	30.6a	31.4a	-	25.6a	-
	Xgwm 261-210	29.5b	-	-	30.2a	31.5a	-	25.6a	-
	Rht-B1b	28.5a	-	-	30.1a	32.0a	-	25.7a	-
	Rht-B1a	28.4a	-	-	30.7a	31.0a	-	25.5a	-
	Xgwm 261-165 + *Rht-B1b*	27.6a	-	-	30.7a	32.0a	-	26.4a	-
	Xgwm 261-165 + *Rht-B1a*	27.3a	-	-	30.5a	30.9a	-	24.9a	-
	Xgwm 261-210 + *Rht-B1b*	29.5b	-	-	29.5a	31.9a	-	25.1a	-
	Xgwm 261-210 + *Rht-B1a*	29.5b	-	-	30.8a	31.1a	-	26.1a	-
Kernel number (kernels head^{-1})	Xgwm 261-165	24.7a	-	-	32.6a	16.7a	-	20.3b	-
	Xgwm 261-210	24.5a	-	-	30.2a	18.2a	-	18.3a	-
	Rht-B1b	24.6a	-	-	32.1a	18.4b	-	19.0a	-
	Rht-B1a	24.5a	-	-	30.8a	16.4a	-	19.7a	-
	Xgwm 261-165 + *Rht-B1b*	24.9a	-	-	33.6b	17.6ab	-	19.5ab	-
	Xgwm 261-165 + *Rht-B1a*	24.4a	-	-	31.7ab	15.8a	-	21.1b	-
	Xgwm 261-210 + *Rht-B1b*	24.3a	-	-	30.6a	19.3b	-	18.4a	-
	Xgwm 261-210 + *Rht-B1a*	24.6a	-	-	29.9a	17.1ab	-	18.3a	-
Heading Date (Julian calendar)	Xgwm 261-165	-	142.3a	-	142.0a	143.4a	143.8a	-	-
	Xgwm 261-210	-	143.3b	-	144.1b	144.8b	145.6b	-	-
	Rht-B1b	-	142.8a	-	143.0a	144.2a	144.7a	-	-
	Rht-B1a	-	142.8a	-	143.1a	144.0a	144.7a	-	-
	Xgwm 261-165 + *Rht-B1b*	-	142.4a	-	142.1a	143.8ab	144.1a	-	-
	Xgwm 261-165 + *Rht-B1a*	-	142.3a	-	141.9a	143.0a	143.6a	-	-
	Xgwm 261-210 + *Rht-B1b*	-	143.2a	-	144.0b	144.5ab	145.4b	-	-
	Xgwm 261-210 + *Rht-B1a*	-	143.3a	-	144.3b	145.1b	145.8b	-	-

Table 2-6. continued.

		Environment							
Trait	Allele	A04†	A05	B05	F04	F05	J04	J05	W05
Above ground Biomass (g m^{-1})	Xgwm 261-165	254a‡	-	-	438a	436a	-	383a	-
	Xgwm 261-210	268a	-	-	423a	461a	-	400a	-
	Rht-B1b	266a	-	-	422a	447a	-	394a	-
	Rht-B1a	257a	-	-	439a	450a	-	389a	-
	Xgwm 261-165 + *Rht-B1b*	259a	-	-	419a	442a	-	388a	-
	Xgwm 261-165 + *Rht-B1a*	249a	-	-	457a	430a	-	377a	-
	Xgwm 261-210 + *Rht-B1b*	272a	-	-	425a	452a	-	400a	-
	Xgwm 261-210 + *Rht-B1a*	265a	-	-	421a	470a	-	402a	-

†Environment abbreviations are as follows: A04, Akron in 2004; A05, Akron in 2005; B05, Burlington in 2005; F04, Fort Collins in 2004; F05, Fort Collins in 2005, J04, Julesburg in 2004; J05, Julesburg in 2005; W05, Walsh in 2005.

‡ For each trait within a column, means followed by the same letter do not differ significantly at α = 0.05 according to the LSMEANS option of SAS PROC GLM.

Rht-B1b allele. The disparity of explained phenotypic variance between the LA, LY and the spring wheat RILs from Butler et al. (2005) illustrates the impact of modifying genes for plant height along with varying production conditions from environment to environment.

No differences in grain yield were observed at any environment between the RILs carrying the Xgwm 261-165 bp and RILs carrying the Xgwm 261-210 bp allele (Table 2-6). Similarly no differences for grain yield were observed between the RILs carrying the *Rht-B1b* and the RILs carrying the *Rht-B1a* allele except under irrigation at Fort Collins in 2004, where RILs carrying the *Rht-B1b* allele (4529 kg ha^{-1}) had higher grain yield than RILs carrying the *Rht-B1a* allele (4165 kg ha^{-1}). These results differ with Butler et al. (2005) who reported differences in grain yield at three of the four environments for RILs carrying the *Rht-B1b* allele compared with the RILs carrying the *Rht-B1a* allele.

No significant differences in test weight were observed between the RILs carrying the Xgwm 261-165 bp allele and RILs carrying the Xgwm 261-210 bp allele, except at Julesburg in 2005 where RILs carrying the Xgwm 261-165 bp (734 kg m^{-3}) had higher test weight than RILs carrying the Xgwm 261-210 bp allele (722 kg m^{-3}) (Table 2-6). Differences ($P \leq 0.05$) for test weight were observed between the RILs carrying the *Rht-B1b* allele and RILs carrying the *Rht-B1a* allele at Fort Collins, Julesburg (2004), and Walsh (2005) where RILs carrying the *Rht-B1a* allele had higher test weights compared with RILs carrying the *Rht-B1b* allele. These results were not as consistent as those reported by Butler et al. (2005) who observed higher test weights at all environments for the RILs carrying the *Rht-B1a* allele compared with RILs carrying the *Rht-B1b* allele.

Differences ($P \leq 0.05$) in kernel weight were observed between the RILs carrying the Xgwm 261-165 bp allele and RILs carrying the Xgwm 261-210 bp allele at one environment (A04) (Table 2-6). At Akron in 2004, RILs carrying the Xgwm 261-210 bp allele had greater kernel weight compared with RILs carrying the Xgwm 261-165 bp allele. No differences in kernel weight were observed between the RILs carrying the *Rht-B1b* allele and RILs carrying the *Rht-B1a* allele. These results differ from Butler et al. (2005) and Flintham et al. (1997) who reported lower kernel weight for lines carrying the *Rht-B1b* allele than lines carrying the *Rht-B1a* allele.

Differences ($P \leq 0.05$) for kernel number were observed between the RILs carrying the Xgwm 261-165 bp allele and RILs carrying the Xgwm 261-210 bp allele at only one environment (J05) (Table 2-6). At Julesburg in 2005, RILs carrying the Xgwm 261-165 bp allele had higher kernel number compared with RILs carrying the Xgwm 261-210 bp allele. Differences ($P \leq 0.05$) for kernel number were observed between the RILs carrying the *Rht-B1b* allele and RILs carrying the *Rht-B1a* allele at only one environment (F05). At Fort Collins 2005, RILs carrying the *Rht-B1b* allele had greater kernel number compared with RILs carrying the *Rht-B1a* allele. These results corroborate those reported by Flintham et al. (1997) who observed greater kernel numbers in lines carrying the *Rht-B1b* allele compared with the lines carrying the *Rht-B1a* allele.

Differences ($P \leq 0.05$) in heading date were observed between the RILs carrying the Xgwm 261-165 bp allele and RILs carrying the Xgwm 261-210 bp allele at Fort Collins and Julesburg in 2004 and Akron and Fort Collins in 2005, where RILs carrying the Xgwm 261-210 bp allele were later heading compared with the RILs carrying the Xgwm 261-165 bp allele (Table 2-6). No differences for heading date were observed between the RILs carrying the *Rht-B1b* allele and the RILs carrying the *Rht-B1a* allele. These results differ from Butler et al. (2005) who reported earlier heading for RILs carrying the *Rht-B1b* allele compared with RILs carrying the *Rht-B1a* allele at three of the four environments.

No differences in above ground biomass were observed between the RILs carrying the Xgwm 261-165 bp allele and RILs carrying the Xgwm 261-210 bp allele (Table 2-6). Similarly, no differences in above ground biomass were observed between the RILs carrying the *Rht-B1b* allele and RILs carrying the *Rht-B1a* allele. These results differ from Butler et al. (2005) who reported lower above ground biomass for RILs carrying the *Rht-B1b* allele than RILs carrying the *Rht-B1a* allele at three of the four environments.

Interaction among the Xgwm 261 and *Rht-B1* alleles

Simultaneous segregation of alleles at the Xgwm 261 and *Rht-B1* loci allowed estimation of interaction effects between alleles at the two loci. Differences ($P \leq 0.05$) in GA sensitivity and coleoptile length were observed among RILs carrying the Xgwm 261-165 + *Rht-B1b* and Xgwm 261-210 + *Rht-B1b* alleles and RILs carrying the Xgwm 261-165 + *Rht-B1a* and Xgwm 261-210 + *Rht-B1a* alleles (Table 2-3). RILs carrying the *Rht-B1b* allele were less sensitive to GA and had shorter coleoptiles than RILs carrying the *Rht-B1a* alleles. These results suggest that the Xgwm 261 locus did not affect GA sensitivity and coleoptile length.

Differences ($P \leq 0.05$) in plant height were observed among RILs with different alleles at the Xgwm 261 and *Rht-B1* loci (Table 2-6). RILs carrying the Xgwm 261-165bp + *Rht-B1b* alleles had the shortest plant height, while RILs carrying the Xgwm

261-210bp + *Rht-B1b* alleles had similar plant heights except at Fort Collins and Julesburg in 2005, where plant heights were greater. RILs carrying the Xgwm 261-165bp + *Rht-B1a* allele had greater plant height than the Xgwm 261-165bp + *Rht-B1b* and Xgwm 261-210bp + *Rht-B1b* allelic classes. RILs carrying the Xgwm 261-210bp + *Rht-B1a* allele had the greatest plant heights across the dryland and irrigated environments.

Differences in grain yield were observed among the RILs carrying the Xgwm 261 + *Rht-B1* allele except at Fort Collins 2004 (Table 2-6). At Fort Collins in 2004, RILs carrying the Xgwm 261-165 bp + *Rht-B1b* and Xgwm 261-210 bp + *Rht-B1b* alleles had greater grain yield than RILs carrying the Xgwm 261-165 bp + *Rht-B1a* and Xgwm 261-210 + *Rht-B1a* alleles. Increased grain yields for lines carrying the *Rht-B1b* allele have been reported for higher yielding environments, such as the Fort Collins 2004 environment (Allan, 1989; Flintham et al., 1997; Butler et al., 2005).

Differences ($P \leq 0.05$) in test weight were observed among the RILs at Akron and Julesburg in 2004 and Julesburg and Walsh in 2005 (Table 2-6). RILs carrying the Xgwm 261-210 bp + *Rht-B1a* alleles had higher test weights compared with RILs carrying the Xgwm 261-165 + Rht-B1b and Xgwm 261-210 + *Rht-B1a* alleles. RILs carrying the Xgwm 261-210 + *Rht-B1b* allele had the lowest test weight than the other allelic classes.

Differences ($P \leq 0.05$) in kernel weight were observed among the RILs carrying the Xgwm 261 + *Rht-B1* loci Akron 2004 (Table 2-6). At Akron in 2004, RILs carrying the Xgwm 261-210 bp + *Rht-B1b* and Xgwm 261-210 + *Rht-B1a* alleles had higher kernel weight compared with RILs carrying the Xgwm 261-165 bp + *Rht-B1b* and Xgwm 261-165 bp + *Rht-B1a* alleles. However, differences in kernel number were observed among the RILs carrying the Xgwm 261 + *Rht-B1* loci at three environments Fort Collins in

2004 and Fort Collins and Julesburg in 2005 (Table 2-6). At these three environments, RILs carrying the Xgwm 261-165 bp + Rht-B1b alleles had greater kernel number than RILs carrying the other allelic classes. These results are opposite those for kernel weight suggesting that when RILs contained fewer kernels, kernel weight was increased.

Differences ($P \leq 0.05$) in heading date were observed among the RILs at Fort Collins and Julesburg in 2004 and Akron and Fort Collins in 2005 (Table 2-6). RILs carrying the Xgwm 261-210 bp + *Rht-B1b* and Xgwm 261-210 + *Rht-B1a* alleles had later heading dates than the RILs carrying the Xgwm 261-165 bp + *Rht-B1b* and Xgwm 261-165 bp + *Rht-B1a* alleles. No differences in above ground biomass were observed among the RILs at Akron and Fort Collins in 2004 and Fort Collins and Julesburg in 2005.

CONCLUSIONS

Wheat cultivars with long coleoptiles as well as short plant height offers growers higher grain yield by improving stand establishment under dry planting conditions characteristic of eastern Colorado and by favoring partitioning of assimilates to grain versus biomass production. Longhorn was used as the common parent in the development of two RIL populations because it is a semi-dwarf, GA sensitive, and has a long coleoptile. Longhorn's Yugoslavian parentage (known source of the *Rht8* gene) suggests it might carry the *Rht8* dwarfing gene. We showed that Longhorn does not carry the *Rht8* semi-dwarfing gene as PCR analysis revealed that Longhorn carries the *Rht-B1a* allele instead of the *Rht-B1b* allele and has the Xgwm 261-210 bp allele instead of the Xgwm 261-192 bp allele associated with *Rht8*. This was the first field study to examine the effects of allelic variation at the Xgwm 261 and *Rht-B1* loci on GA sensitivity, coleoptile length and agronomic traits. In the LY RIL population, greater sensitivity to GA and longer coleoptile length was observed in RILs carrying the *Rht-B1a* allele than the RILs carrying the *Rht-B1b* allele regardless of the Xgwm 261 allele. The RILs carrying the Xgwm 261-210 bp and the *Rht-B1a* allele were similar to Longhorn for GA sensitivity and coleoptile length. However, compared to Longhorn, taller plant height was observed in the RILs carrying the Xgwm 261-210 bp and the *Rht-B1a* allele. Longhorn with its intermediate plant height, may not have been the best parental choice and our use of this cultivar may explain some of the contradictory results in this study. The use of microsatellite markers to identify the Xgwm 261-210 bp and the *Rht-B1a* allele does not guarantee greater coleoptile length and semi-dwarf plant height. Therefore the use of these markers must be accompanied by coleoptile length, GA sensitivity, and plant height measurements to ensure the development of semi-dwarf cultivars with the desired coleoptile length and plant height.

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