CHARACTERIZATION AND TESTING OF *REDUCED HEIGHT (RHT*) HYPOMORPHS IN DURUM AND SPRING WHEAT

by

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DEDICATION

To my parents for their constant support and enthusiasm for all of my accomplishments and for teaching me the importance of hard work, responsibility, and kindness.

To my brother for always answering my calls on my walks back home from long days in the lab or field and never failing to make me chuckle.

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ABSTRACT

The Reduced Height (*Rht*) gene in wheat (*Triticum aestivum L.*) increases yield by partitioning less nutrients to stem elongation and more towards spike development. In hexaploid wheat, the mutations *Rht-B1b* and *Rht-D1b,* create high-yielding semi-dwarf varieties. While *Rht-B1b* and *Rht-D1b* have been widely adopted due to their ability to increase yield, they also have drawbacks such as smaller seed size and lower protein content. Furthermore, tetraploid durum wheat (*Triticum. turgidum L*), *Rht-B1b* creates plants that are shorter than in hexaploid wheat under Northern Great Plains growing conditions. This project aimed to further characterize *Rht* and to develop a plant height intermediate between current standard-height and semi-dwarf varieties to increase yield in both durum and spring wheat. To create novel Rht alleles, seeds were mutagenized with Ethyl-methanesulfonate (EMS) and mutations were identified. Near-isogenic lines (NILS) were developed for the two *Rht-A1* alleles and *Rht-B1b-E529K* alleles in semi-dwarf (*Rht-B1b*) and standard height (*Rht-B1a*) varieties in durum. In spring wheat, NILs were developed for eight Rht-A1 alleles in two high-yielding Montana varieties. These NILS were planted in field trials and plant height and grain traits were measured. Four novel mutations, *Rht-A1-E63K*, *Rht-A1-Q6**, *Rht-A1-V55M*, and *Rht-A1-53T* in spring wheat and two mutations in durum, *Rht-B1b-E529K* and *Rht-A1-S50F* all had either significantly changed height or grain traits. Along with developing and testing Rht alleles for field trait improvement, we did a study to characterize an Rht stop-codon dosage response in wheat. Previous studies in rice and barley have indicated that a lack of the functional SLR1/SLN1 gene respectively, results in an abnormal growth response characterized by taller height and *slender* appearance. This effect on *Rht* function has yet to be tested in wheat. *Rht* nonsense alleles were created by screening an EMS treated population created using seed of a standard-height Montana variety. We combined mutations creating lines homozygous for single, double, or triple mutations. In field trials, *Rht* triple mutants exhibited a slender, elongated phenotype with strike heads similar to *SLN1* mutants in barley. Differences in height varied for the other crosses but did trend towards increased height with increased *Rht*-stop mutation dosage.

INTRODUCTION

Reduced Height (Rht) Background and History

The *Reduced Height* gene (*Rht*) plays a crucial role in regulating plant height and tiller development in wheat (*Triticum aestivum L.*). Mutations of the *Rht* gene have been widely used in agriculture to produce shorter, more compact wheat plants that increase productive tillers and yield. Molecular characteristics of *Rht* have been well-described. *Rht* encodes a DELLA (Asp-Glu-Leu-Leu-Ala) protein, which acts as a negative regulator of gibberellic acid (GA) response (Zentella, et al, 2007.). Gibberellic acid (GA) is a phytohormone responsible for regulating seed germination and stem elongation (Hauvermale, et al. 2012). In the presence of GA, DELLA proteins are bound by *GA-Insensitive Dwarf 1 (GID1*) which is a GA receptor protein (Shimada et al. 2008). They are then polyubiquinated using the E3 ubiquitin ligase complex which is then degraded by the 26S proteasome (Griffiths et al., 2007; Lou et al. 2016; M. Ueguchi-Tanaka et al. 2005). The DELLA protein degradation results in the plant responding to GA and continuing to grow taller. If GA is absent, DELLA proteins are not degraded, and GA responses and plant growth is repressed.

Mutations near the DELLA motif can alter plant growth and development. In wheat, *Rht* is responsible for encoding DELLA proteins. There is a single functional copy of *Rht* on each of the group four chromosomes (Gale et al.1975; McVittie et al. 1978; Sourdille et al., 1998). Though there have been many semi-dwarfing genes characterized in wheat, *Rht-B1b* and *Rht-D1b* are the most common semi-dwarfing alleles. (Allan, 1970; Allan, Vogel, & Craddock, 1959; Gale & Gregory, 1977). The position of the *Rht-B1b* and *Rht-D1b* mutations only differs by one amino acid and are functionally indistinguishable (Flintham et al 1997; Peng et al. 1999). *Rht-*

B1b and *Rht-D1b* both introduce a premature stop codon into *Rht* that partially suppresses gibberellic acid GA response leading to a semi-dwarf phenotype. Both decrease hexaploid wheat height by about 20% and increase yield by about 21% under conditions favorable to yield increases conditioned by additional tillers (Flintham et al., 1997; Hoogendoorn et al. 1990). Semi-dwarf varieties containing *Rht-B1b* or *Rht-D1b* are also less susceptible to lodging under increased water and nitrogen inputs due to their decreased height (Hedden, 2003).

The use of *Rht-B1b* and *Rht-D1b* alleles in wheat breeding was a major contributor to the Green Revolution in the mid-20th century, which saw a significant increase in food production and food security worldwide. *Rht-B1b* and *Rht-D1b* were discovered in the same Japanese wheat landrace and were then crossed into two American wheat cultivars to create an improved semi-dwarf variety, Norin 10 (Lumpkin, 2015). Norin 10 was then crossed with other wheat varieties and became a part of the semi-dwarfing germplasm used and promoted by Norman Borlaug to increase agricultural yield (Borlaug, 1968; Reynolds & Borlaug, 2006). *Rht-B1b* or *Rht-D1b* are now found in at least 70 % of all modern hexaploid wheat cultivars (Evans, 1998).

Limitations of *Rht-B1b* and *Rht-D1b*

Despite the advantages of *Rht-B1b* and *Rht-D1b*, these mutations do have negative pleiotropic effects. *Rht-B1b* and *Rht-D1b* decrease coleoptile length which can reduce seedling emergence, particularly in arid climates.(Fick, 1976; Schillinger et al. 1998). *Rht-B1b* and *Rht-D1b* also reduce seed protein content and size (N.E. Appleford et al., 2007; Casebow et al., 2016; Gale & Youssefian, 1985). Furthermore, in durum wheat, (*Triticum turgidum* ssp. *durum)* these pleiotropic effects are compounded. This is because durum is a tetraploid with only the A and B genome and the plant height, seed protein content and size reductions are greater in durum than in bread wheat (Mathews et al., 2006). Though *Rht* has been studied for decades, there are still opportunities to expand our knowledge on how *Rht* functions and to determine whether creating new *Rht* alleles may lead to practical improvements.

Currently, no semi-dwarfing *Rht-A1* alleles have been discovered despite its expression levels being similar to those of *Rht-B1* and *Rht-D1* (Jobson et al 2020; Pearce et al. 2011). The creation and characterization of alternative *Rht* alleles could provide new allelic diversity and perhaps reduce pleiotropic effects associated with the *Rht-B1b* and *Rht-D1b* mutations.

Chapters one and three describe projects that focus on the creation and testing of *Rht-A1* alleles. Chapter one specifically looks at the incorporation of the durum *Rht-A1* alleles previously described in Brown et al. (2022) and the durum *Rht-B1b-E529K* allele previously described in Mo et al. (2018) into Montana-adapted near-isogenic lines. In chapter three, eight novel *Rht-A1* alleles were tested in two Montana-adapted spring wheat varieties. These field trials include a range of agronomic measurements.

Along with creating new *Rht* alleles to incorporate into adapted cultivars we also aimed to determine a *Rht* dosage response in hexaploid wheat. The impact of stop mutations in genes heterologous to *Rht* in other plants such as *Slender 1* (SLN1) in barley, *Slender 1* (SLR1) in rice, and RGA and GAI in *Arabidopsis* have all been characterized but this response had not previously been documented in wheat (Brown et al., 2022; P. M. Chandler et al., 2008; Ikeda, Ueguchi-Tanaka et al., 2001; J. Peng et al., 1997; Wilhelm et al. 2013; Winkler, 1994). In chapter two, we describe how we combined *Rht-A1*, *Rht-B1*, and *Rht-D1* stop mutations to create an *Rht* dosage series that we tested by measuring coleoptiles and plant growth under field conditions. All of the work contained in this thesis aimed to further our understanding of how

Rht impacts plant growth and development and whether that understanding can be used to improve agronomic yield in wheat.

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CHAPTER ONE:

EXPANDING *REDUCED HEIGHT* (*RHT*) ALLELIC DIVERSITY IN DURUM (*TRITICUM TURGIDUM L.*) WHEAT VIA CREATION AND TESTING OF *RHT* HYPOMORPHS

Introduction

As global population continues to increase while productive land becomes more limiting, new strategies to increase yield are a focus in many breeding programs. One of the ways that yield has been increased in hexaploid wheat (*Triticum aestivum* L.) is through mutations in the *Reduced Height* genes *Rht-B1* and *Rht-D1* on chromosome 4B and 4D, respectively that encode *DELLA* proteins (Gale & Marshall, 1973; J. Peng et al., 1999; Youssefian et al. 1992). In the presence of gibberellic acid (GA), the GA receptor, *Gibberellin Insensitive Dwarf1* (*GID1*) binds to wild type *DELLA* proteins resulting in their ubiquitination and degradation through the 26S proteasome *SCF* complex (Pearce et al., 2011; J. Peng et al., 1999). The naturally occurring dominant acting and semi-dwarfing mutant alleles, *Rht-B1b* and *Rht-D1b* contain a premature stop codon in the N-terminus of the *DELLA* domain that prevents the *GID1* receptor from binding (Peng et al. 1999). After the stop codon in *Rht-B1b* and *Rht-D1b*, Rht translation reinitiates resulting in N-terminally truncated *DELLA* proteins (Mo, 2018). Since the *DELLA* proteins are shortened, they cannot detect GA signals mediated by *GID1* rendering the plant partially insensitive to GA resulting in a semi-dwarf phenotype (Pearce et al., 2011).

Rht-B1b and *Rht-D1b* have been widely incorporated into hexaploid wheat varieties since the 1960’s contributing to higher grain yields by allowing plants to tolerate higher nitrogen inputs without lodging (Gale, Marshall, & Rao, 1981). Furthermore, decreasing stem height allowed for more assimilate partitioning into developing heads which increases the harvest index (Youssefian et al., 1992). This resulted in yield increases of about 20 % (Flintham et al., 1997).

Though well characterized in hexaploid wheat, the effects of *Rht* mutant alleles on durum wheat (Triticum. turgidum L. supsp. Durum (Desf.) are lesser studied. Durum wheat is the tetraploid wheat species grown for milling into semolina, used in pasta and couscous production. In the United States, Montana and North Dakota are the top two producers of durum. Montana grew 618,359 hectares in 2021 worth 450 million US dollars (National Agricultural Statistics Service, 2021).

Though the B genome contains a mutant *Rht-B1b* allele in many semi-dwarf hexaploid wheats, the A and D genome still contain wild-type *Rht* genes which results in a height reduction of about 21% (Flintham et al., 1997; Hoogendoorn et al., 1990). Current *Rht* mutant alleles impose greater challenges in tetraploid wheat compared to hexaploid wheat. The height reduction effects of *Rht-B1b* are amplified in durum wheat due to the lack of a D genome and thus only the A genome contains a wild-type *Rht* gene resulting in a height decrease of up to 45% (Mathews et al., 2006). A height reduction this great can result in plants too short for effective mechanical harvest. Furthermore, *Rht-B1b* reduces coleoptile length in durum (Pandey et al., 2015). If coleoptiles are short, poor emergence may occur in dryland environments where crops are seeded deep into moisture (Schillinger et al., 1998). Along with decreasing height and coleoptile length, *Rht-B1b* also decreases seed size and protein content (Amram et al., 2015; Brown et al., 2022; Fick, 1976; E.M. Jobson, Martin, Schneider, & Giroux, 2018; Liatukas, 2011).

Other *Rht-B1* and *Rht-D1* alleles have been researched in hexaploid wheat but no *Rht-A1* mutations have been found to significantly decrease height in hexaploid wheat. However, the expression levels of Rht-A1 is similar to Rht-B1 in stem tissue (Pearce et al., 2011). This led to the hypothesis that specific *Rht-A1* mutations in durum could modify plant height; and that creating hypomorphic or partially functional *Rht-A1* alleles could result in plant height intermediate between *Rht-B1b* and *Rht-B1a*. Similarly, *Rht-B1b-E529K* in relation to *Rht-B1b* increased plant height by 21% as well as coleoptile length indicating the potential for an intermediate height phenotype in durum (Mo et al. 2018).

Brown et al. (2022) described the creation of two *Rht-A1* missense mutations from an EMS population in the durum variety ‘Divide’ Elias & Manthey, 2007) (PI 642021) *(Rht-A1-S50F* and *Rht-A1-L358F)*. These two mutants along with *Rht-B1b-E529K* (Mo, 2018) were crossed to both semi-dwarf (*Rht-B1b*) and tall (*Rht-B1a*) varieties and tested in space-planted, multi-environment field trials using BC_1F_2 plants. The results showed that *Rht-B1b-E529K* gave an intermediate plant height phenotype as it increased plant height in the semi-dwarf (*Rht-B1b*) background relative to *Rht-B1b*, and in the standard height (*Rht-B1a*) background it decreased plant height. These field trials demonstrated that *Rht-A* mutations did not significantly affect plant height in the presence of *Rht-B1b*. However, the *Rht-A1-S50F* mutation decreased plant height when combined with *Rht-B1a*. Other agronomic traits were not affected by the tested *Rht-A1* mutations under the space planted conditions used (Brown et al., 2022).

Yeast-2-hybrid assays were conducted to test the *Rht-A1* mutant alleles and *Rht-B1b-E529K* responsiveness to GA (Brown.et al, 2022). The results showed that *Rht-A1a* and *Rht-B1a* interacted with GID1 in the presence and absence of GA. The *Rht-B1b-E529K* mutation also showed no interaction as expected since the *Rht-B1b* stop codon occurs in the protein prior to the E529K missense change and translation re-initiation that happens *in planta* does not occur in yeast. This is an indication that the *in-vitro* effect of *Rht-B1b* is not affected by the addition of the *E529K* mutation. The *Rht-A1-S50F* missense mutation showed no interaction in the absence of GA but did interact to a similar degree as *Rht-B1a* in the presence of GA. *Rht-A1-L38F* showed a weak interaction in the absence of GA and normal binding to GID1 in the presence of GA.

A coleoptile experiment was conducted to further test GA response and determine *Rht* mutation impacts on coleoptile length (Brown et al. 2022). The results of the tests showed that the *Rht-B1b-E529K* mutation significantly increased coleoptile length relative to *Rht-B1b* and decreased length compared to *Rht-B1a*. The effect of the *Rht-B1b E529K* mutation was greater in the presence of GA compared to water. There was no significant difference in coleoptile length in either *Rht-A1* allelic group. These findings were consistent with other studies that cite *Rht-B1b* and *Rht-D1b* as being the only *Rht* mutations that impact coleoptile length (Brown et al., 2022; Fick, 1976; Jobson et al. 2019; Liatukas, 2011; Schillinger et al., 1998)

These initial results indicate that these *Rht* mutant alleles could be used to create intermediate height durum varieties. These results however are limited, as Brown et al. (2022) used spaced-plant trials which are not representative of typical field practices in which plants are seeded at much higher density. Furthermore, the plants used in these previous trials from Brown et al. (2022) were not near-isogenic lines and therefore other unknown genetic interactions could have influenced results. The present study considers the effects of two *Rht-A1* alleles and the

Rht-B1b-E529k allele in near-isogenic line (NIL) durum varieties planted both in spaced-planted and full density trials.

Methods

NIL Development

The three alleles (*Rht-A1-S50F, Rht-A1-L358F, and Rht-B1b-E529K*) were crossed into the recurrent parents, Lustre, a standard height (PI 695072) (Hogg et al., 2022) and MTD2219 (semi-dwarf) followed by backcrossing to the recurrent parents for four generations and then selfing for two generations to create BC_5F_2 derived NILs.

To determine genotypes at each generation, leaf tissue was collected from individual 2-week-old seedlings and DNA was extracted as previously described by Riede and Anderson (1996). The DNA was used in PCR reactions using the nested approach of Li et al. (2013) utilizing *Rht* A- or B- genome-specific primers to amplify the *Rht-A1* or *Rht-B1* coding sequence and then internal primers were used for amplification of the region containing the *Rht-A1-S50F* and *Rht-A1-L358F* mutations. To detect the *Rht-B1b-E529K* mutation, the internal primers from Mo et al. (2018) were used. The PCR products were Sanger sequenced (GENEWIZ, Inc. Burlington, MA) then the sequence was compared to the *Rht-A1* reference accession (JF9302277) and the *Rht-B1* reference accession, (JF930278) (www.ncbi.nlm.nih.gov) using the DNASTAR SeqMan Pro software (DNASTAR version 15.0.1.1, DNASTAR Inc. Madison, WI). Plants fixed for *Rht-B1a* and *Rht-B1b,* but heterozygous for the *Rht-A* mutations and *Rht-B1b-E529K* were kept for planting in field trials. In the last backcross generation, heterozygous plants were allowed to self –pollinate and BC_5F_2 plants homozygous for the alternative alleles were selected for seed increase and subsequent field testing. Comparisons between *Rht* mutant and wild-type lines were derived from a single *Rht-A1* or *Rht-B1* heterozygous plant in the last backcross generation.

Field trials

Field trials were conducted at the Arthur H. Post Agronomy Farm near Bozeman, MT (45.67° N, 111.15° W, 1,455 m above sea level). The soil is an Amsterdam silt loam (Fine-silty, mixed, superactive, frigid Typic Haplustolls) with a pH of 6.5. Before planting, 289 kg/ha of Urea (46-0-0) was applied. One hundred seeds from each of the six crosses were hand-sown to a depth of 2.5 cm on 19 May 2021 in 3-m rows with 30-cm spacing between each row. Seeds were planted 15 cm apart with 20 plants per row. From 1 May to 31 August, the research station received 17.2 cm of precipitation. The highest recorded air temperature was 37.2° Celsius on 8 August 2021 and the lowest recorded air temperature was -4.4°Celsius on 22 May 2021 (https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00241047/detail). Five cm of irrigation was applied using hand line sprinklers on 22 June, 28 June, and 5 July. On 6 June, 1.75 l/ha of Vendetta (3,5-dibromo-4-Hydroxybenzonitrile 31.7%, 2-Ethylhexyl ester of 2-methyl-chlorophenoxyacetic acid 34% Wilbur-Ellis Co. Fresno, CA USA) and 0.77 l/ha of Parity (Fenoxaprop-P-Ethyl 11.3% Tenkoz Inc. Alpharetta, GA USA) were applied for disease/weed control. Plants were genotyped using the methodology described above and only *Rht* homozygous plants were considered for measurement and harvest. Each single-plant provided $BC_5F_{2:3}$ seed that was planted in Yuma, AZ in October 2021 for seed increase.

In 2022, $BC_5F_{2:4}$ seed for each of the six crosses were planted in separate rain fed and irrigated experiments at the Arthur H. Post Agronomy farm. Each experiment had two replicates arranged in randomized complete block split plot design. The six backcrosses were main plots and the individual NILs within each backcross population were subplots. Plots consisted of four 3-m rows spaced 30 cm apart. Seeds were mechanically sown to a depth of 5 cm on 17 May 2022. The irrigated experiment received 5 centimeters of water applied using hand line sprinklers on 21 June, 26 June, and 2 July. From 1 May to 31 August, the research station received 19.8 cm of precipitation. On 3 June, 0.05 l/ha of Affinity Tank Mix (Thifensulfuron-methyl 40%, Tribenuron methyl 10% E.I. DuPont de Nemours and Co. Wilmington, DE USA), 0.50 l/ha MCPE (2-methyl-4-chlorophenoxyacetic acid isocytl (2-ethylhexyl) ester 68.7%, Agrilliance, LLC. St. Paul MN USA), 1.17 l/ha of Discover (Clodinafop-propargyl 6.4%, Syngenta Crop Protection LLC. Greensboro, NC USA) were applied for weed and disease control. The highest recorded air temperature was 33.8° Celsius on 1 August 2022 and the lowest recorded air temperature was -3.8° Celsius on 9 May 2022 (https://www.ncdc.noaa.gov/cdoweb/datasets/GHCND/stations/GHCND:USC00241047/detail).

Plant Measurements

Plant measurements were taken from single plants in 2021 and from five representative plants within each 4-row plot in 2022. At 10 days post-anthesis, flag leaf length of a representative primary tiller was measured from base of the plant to tip of the head and flag leaf width was measured at the widest point of the leaf. Plant height was measured at physiological maturity as the distance from the soil surface to the terminal spikelet of a representative primary tiller. Peduncle height was also measured at physiological maturity from the base of the topmost node to the terminal spikelet of a representative tiller to the base of the head. Productive tiller number was counted at the base of plants as only tillers with heads that formed seed. At physiological maturity, the five representative plants measured per plant were cut at ground level, bundled, weighed, and threshed with a single-plant thresher. Plant weight was measured as biomass (included the weight of straw plus the weight of grain). Grain protein was analyzed with the LECO-FP 528 (LECO Co. St. Joseph, MI USA) (AACC 2009) and individual seed weight was calculated by weighing a sample of 200 seeds using a seed counter.

Statistical Analysis

Rht allelic class mean comparisons for each cross were made using a one-way analysis of variance (ANOVA) in R (R Foundation for Statistical Computing. Version 4.0.5. Vienna, Austria) for the spaced-plant trial in 2021. Data for the 2022 trials were combined over environments for each cross. The model was as follows:

Response = Environment + replication within environment + genotype class + lines within genotype class + environment x genotype class + environment x lines within genotype class + random error. Individual lines within genotype class and their interaction with environment were considered random and other factors were fixed using the lmer4 package in R (Bates et al. 2015).

Results

2021 Field Trial

The effect of the durum wheat *Rht* mutations were tested in 2021 in BC_5F_2 lines in an irrigated, spaced, single-plant trial. The *Rht-B1b-E529K* cross in the semi-dwarf background (*Rht-B1b*) showed the largest difference in plant height ($P < 0.001$) (Table 1.1). On average, plants with the *Rht-B1b-E529K* allele were 29.7 % taller than those with *Rht-B1b*. The *Rht-B1b-E529K* allele also showed a significant impact on plant height ($P<0.001$) in the standard-height background (*Rht-B1a*) with *Rht-B1b-E529K* mutant plants being on average, 13.9% shorter than those containing *Rht-B1a*. Neither of the novel *Rht-A1* mutations showed significant differences when compared to *Rht-A1a* in the semi-dwarf background. However, the *Rht-A1-S50F* allele did on average, increase plant height by 8.6% (P=0.061) and increase head length by 17% (P=0.063). This is indicative of a strong trend towards increasing overall height as well as on average head length. Differences in grain traits (Table 2.1) and other traits between allelic class means were not detected.

2022 Field Trial

In 2022, $BC_5F_{2:3}$ lines were planted in full density irrigated and dryland plots. Because genotype by environment interactions were infrequent in field traits, values are presented as means averaged over dryland and irrigated environments. *Rht-B1b-E529K* showed significant effects when compared to both *Rht-B1b* and *Rht-B1a*. *Rht-E529*K increased plant height (P<0.001) by 23% and peduncle height (P<0.01) by 16.4% compared to *Rht-B1b* (Table 3.1). *Rht-A1-S50F* also showed significant effects but in the presence of *Rht-B1b* only. *Rht-A1-S50F* increased plant height (P<0.05) by 3.9% and increased productive tillers (P<0.05) by 2% in the presence of *Rht-B1b*.

The *Rht-B1b-E529K* mutant decreased plant height (P<0.001) by 7.6% and peduncle length (P<0.05) by 3.2% compared to *Rht-B1a*. We did detect a genotype by environment interaction for head length in *Rht-B1b-E529K* compared to *Rht-B1a* with a larger difference in the irrigated environment. In the irrigated trial, *Rht-B1b-E529K* and *Rht-B1b* head length means were 7.99 cm vs 7.53 cm (P<0.001) and in the dryland trial were 7.33 cm vs. 7.32 cm, respectively. The *Rht-A1-L358F* mutation appeared to have no effect on head length in either environment.

The *Rht-B1b-E529K* mutant increased thousand kernel weight by 16.4 % (P<0.01) compared to *Rht-B1b* (Table 4.1) and when compared to *Rht-B1a*, *Rht-B1b-E529K* decreased thousand kernel weight by 5.8% (P<0.05). There was no difference among any other grain traits between allele classes for either of the *Rht-A1* mutations.

Discussion

Our field trials demonstrated that *Rht-A1-S50F* and *Rht-B1b-E529K* both impacted growth traits. The previously characterized height effects of *Rht-B1b-E529K* were observed in both the space planted (Table 1.1) and the full-density trial (Table 3.1) (Brown et al., 2022; Mo et al., 2018)

Furthermore, *Rht-B1b-E529K* produced a height-decreasing effect in both the spaced-plant trial in 2021 and full-density trial in 2022 as well as decreased peduncle height in 2022. We expected and observed that the *Rht-B1b-E59K* allele would increase individual seed weight and protein relative to *Rht-B1b* and decrease seed weight relative to *Rht-B1a*. Total grain weight was not significantly different in either background despite the observed increase in thousand kernel weight. This likely could be due to the energetic tradeoff associated with grain traits that are negatively correlated such as grain weight, grain number, and spikelet number (Quintero et al. 2014; Sadras, 2007; Tillett et al. 2022; Xie & Sparkes, 2021).

We did not observe any protein content differences among any of the alleles tested including *Rht-B1b-E529K* which we expected to have higher protein content than *Rht-B1b.* Further testing in more drought-prone environments may allow us to better observe protein differences. Overall, the results observed indicate that *Rht-E529K* can be used to confer an intermediate-height phenotype in durum that is between current semi-dwarf and standard-height varieties while maintaining current yield potential and protein content.

The novel *Rht-A1-S50F* mutation did not have any effect on agronomic traits in the *Rht-B1a* standard-height background but in the presence of *Rht-B1b*, the *Rht-A1-S50F* mutant increased height and productive tiller number in the full seeding density trial in 2022. The spaced plant trial in 2021, however, showed full height not being significantly different from the wild-type though the *Rht-A1-S50F* mutant did show a trend toward increasing plant height. This could be because the conditions of the spaced plant trial were more variable compared to the full density trial in 2022.

Furthermore, the 2021 field season experienced higher instances of drought with less favorable rainfall and higher temperatures compared to 2022, which may have contributed to unknown genotype by year interactions. The *Rht-A1-S50F* mutation did not impact grain traits in either the spaced plant trial in 2021 or the full density trial in 2022 but the scope of this trial is limiting as the environmental conditions of the test site in Bozeman, MT are often more favorable than in other drier regions across the state. Future testing in multiple locations and environments could accentuate possible grain trait differences. The other novel *Rht-A1* allele tested, *Rht-A1-L358F* showed no difference in any traits compared to wild type *Rht-A1* in either background.

Our findings demonstrate that *Rht-B1b-E529K* and *Rht-A1-S50F* both affected agronomic traits and can be utilized to create intermediate height durum wheat. Both *Rht-B1b-E529K* and *Rht-A1-S50F* increased plant height compared to *Rht-B1b* and the *Rht-B1b-E529K* decreased height when combined with *Rht-B1a* in standard height plants. These findings along with increased coleoptile length in both mutations described in Brown et al. (2022) give evidence that incorporating *Rht-B1b-E529K* or *Rht-A1-SF50* mutations into adapted durum wheat lines would benefit certain environments and minimize the disadvantages conferred by current semi-dwarf varieties.

Table 1.1: The impact of *Rht* mutations on genotype class mean values for agronomic field traits from 2021 field trials in single plant trials under irrigated conditions.

***Rht B1* Cross**	***Rht* Mutant Allele**	**n**	**Plant Height[a] cm**	**Head Height[a] cm**	**Tillers[a] No./plant**	**Spikelets[a] No./head**
Rht-B1b	*Rht-B1b-E529K*	9	77.3±1.97***	5.92±0.26	11.9±1.74	14.8±0.60
	Rht-B1b	11	59.6±1.68	6.25±0.22	13.0±1.48	14.1±0.51
	Rht-A1-S50F	7	60.2±2.33	6.67±0.38	14.7±1.73	14.5±0.55
	Rht-A1a	11	55.0±1.81	5.70±0.29	11.2±1.34	15±0.43
	Rht-A1-L358F	13	57.9±0.43	6.02±0.12	13.6±1.21	15.4±0.28
	Rht-A1a	9	57.1±0.59	6.06±0.16	14.9±1.65	14.1±0.38
Rht-B1a	*Rht-B1b-E529K*	10	60.9±1.23***	7.81±0.31	14.8±1.83	17.6±0.33
	Rht-B1a	12	69.4±1.65	8.58±0.41	16.2±2.45	17.4±0.78
	Rht-A1-S50F	10	74.1±1.91	8.02±0.26	13.9±1.71	17.8±0.66
	Rht-A1a	10	75.2±1.91	8.12±0.26	15.1±1.71	17.8±0.66
	Rht-A1-L358F	9	73.6±2.03	8.43±0.32	13.4±2.46	18.7±0.55
	Rht-A1a	10	74.7±1.90	8.54±0.30	16.1±2.30	19±0.51

[a] Values represent the average for each genotype ± the standard error.

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

Table 2.1: The impact of *Rht* mutations on genotype class mean values for agronomic grain traits from 2021 field trials in single plant trial under irrigated conditions.

Rht B1 **Cross**	***Rht*** **Mutant Allele**	**n**	**Grain Weight[a] g/plant**	**Plant Weight[a] g/plant**	**Harvest Index[a] %**
Rht-B1b	*Rht-B1b-E529K*	9	15.3±2.27	34±4.3	42.8±1.89
	Rht-B1b	11	17.2±1.93	37.4±3.67	45.8±1.61
	Rht-A1-S50F	7	25.2±4.83	53.3±8.9	46.2±1.05*
	Rht-A1a	11	18.2±3.74	35.4±6.9	48.5±8.16
	Rht-A1-L358F	13	16±1.92	41.5±3.93	36.7±3.97
	Rht-A1a	9	16.8±2.72	36.8±6.34	52.2±6.41
Rht-B1a	*Rht-B1b-E529K*	10	20.5±4.25	71.8±12.6	30.6±2.78
	Rht-B1a	12	26±5.7	77±16.9	30.9±3.72
	Rht-A1-S50F	10	22.7±3.35	57.1±8.18	40.0±3.45
	Rht-A1a	10	24.2±3.55	59.2±818	34.5±3.45
	Rht-A1-L358F	9	21.2±3.72	65.3±11.1	33.1±3.10
	Rht-A1a	10	25.8±3.48	66.4±10.4	40.3±2.90

[a] Values represent the average for each genotype ± the standard error.

*, **, and *** denote P values <0.05, 0.01, and 0.001, respectively.

Table 3.1: The impact of *Rht* mutations on genotype class mean values for agronomic traits from 2022 full density field trials averaged over two replications in irrigated and dryland.

Rht B1 **Cross**	***Rht*** **Mutant Allele**	**Plant Height[a] cm**	**Peduncle Height[a] cm**	**Head Height[a] cm**	**Productive Tillers[a] No/15cm^2**	**Flag Leaf Length[a] cm**	**Flag Leaf Width[a] cm**
Rht-B1b	*Rht-B1b-E529K*	80.5±2.47***	38.3±1.36**	6.11±0.09	33±1.28	18.7±0.55	1.38±0.03
	Rht-B1b	65.4±2.15	32.9±1.16	6.07±0.07	33.7±1.14	18.9±0.53	1.40±0.04
	Rht-A1-S50F	62.6±0.80*	30.3±0.69	6.28±0.10	33±1.28*	18.8±0.71	1.38±0.01
	Rht-A1a	60.2±0.75	30.1±0.63	6.11±0.10	33.7±1.14	18.2±0.66	1.4±0.02
	Rht-A1-L358F	60±0.54	28.7±0.21	6.13±0.10	31.8±1.45	22.8±0.50	1.59±0.04
	Rht-A1a	59.5±0.64	28.5±0.32	6.2±0.12	30.8±1.45	22.5±0.50	1.52±0.06
Rht-B1a	*Rht-B1b-E529K*	79±1.71***	34±0.63*	7.43±0.02**	37.2±2.11	21.9±0.42	1.49±0.03
	Rht-B1a	85.5±1.76	35.1±0.66	7.65±0.21[b]	34.7±2.27	22.3±0.42	1.49±0.04
	Rht-A1-S50F	84.9±2.17	35±0.52	7.9±0.17	31.8±1.45	22.4±0.41	1.59±0.04
	Rht-A1a	84.3±2.22	34.7±0.52	7.79±0.17	30.8±1.45	22.5±0.41	1.52±0.04
	Rht-A1-L358F	85.8±0.60	35.8±0.51	7.44±0.14	30.2±5.76	23.5±0.42	1.6±0.06
	Rht-A1a	86.9±0.60	35.8±0.50	7.63±0.14	32.5±5.76	23.2±0.42	1.57±0.06

[a] Values represent the average for each genotype ± the standard error.

[b] Genotype by environment interaction found to be significant at a P-value of 0.01 level.

n=16 values in each genotype group, each value representing the average of five plants each in two replicates.

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

Table 4.1: The impact of *Rht* mutations on genotype class mean values for agronomic grain traits from 2022 full density field trials averaged over two replications in irrigated and dryland.

Recurrent Parent *Rht* Allele	***Rht* Mutant Allele**	**Thousand Kernel Weight[a] mg/seed**	**Grain Weight[a] kg/0.0004 ha**	**Protein Content[a] %**
Rht-B1b	*Rht-B1b-E529K*	38.3±1.36**	1.52±0.04	14.9±0.15
	Rht-B1b	32.9±1.16	1.59±0.04	14.7±0.13
	Rht-A1-S50F	30.3±0.69	1.33±0.06	15.2±0.19
	Rht-A1a	30.1±0.63	1.42±0.05	14.7±0.17
	Rht-A1-L358F	28.7±0.21	1.62±0.05	14.2±0.16
	Rht-A1a	28.5±0.32	1.62±0.06	14.4±0.18
Rht-B1a	*Rht-B1b-E529K*	34.0±0.63*	1.84±0.06	14.8±0.07
	Rht-B1a	36.1±0.66	1.86±0.06	15.0±0.08
	Rht-A1-S50F	35.0±0.52	1.67±0.10	15.6±0.09
	Rht-A1a	34.7±0.52	1.65±0.10	15.6±0.09
	Rht-A1-L358F	35.8±0.51	1.83±0.03	15.3±0.14
	Rht-A1a	35.8±0.61	1.80±0.03	15.4±0.14

[a] Values represent the average for each genotype ± the standard error.

n=16 values in each genotype group, each value representing the average of five plants each in two replicates.

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

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CHAPTER TWO:

WHEAT (*TRITICUM AESTIVUM L.*) *REDUCED HEIGHT* (*RHT*) DOSAGE SERIES REVEALS *RHT* IMPORTANCE IN WHEAT DEVELOPMENT

Introduction

Gibberellic acid (GA) is a plant hormone essential for plant growth that plays a major role in regulating developmental processes. GA responses are regulated by DELLA (Asp-Glu-Leu-Leu-Ala) family proteins. In the presence of GA, DELLA proteins are bound by *GA-Insensitive Dwarf 1 (GID1*) which is a GA receptor protein (Shimada et al., 2008). They are then polyubiquinated using the E3 ubiquitin ligase complex which are then degraded by the 26S proteasome (Griffiths et al., 2007; Lou et al., 2016; M. Ueguchi-Tanaka et al., 2005; M. Ueguchi-Tanaka et al. 2007). This DELLA protein degradation causes the plant to respond to the GA signal. If GA is absent, DELLA proteins are not degraded, and GA responses are repressed.

DELLA proteins contain two domains, the N-terminal DELLA domain, and the C-terminal GAI, RGA, and SCARECROW (GRAS) domains. The N-terminal DELLA domain is characterized by the DELLA motif (which is involved in the interaction between GID1 and DELLA), the TVHYNP motif, and the poly S/T/V motif (Ikeda et al., 2001; Itoh et al. 2002; Murase et al. 2008; Silverstone et al. 1998). The C-terminal GRAS domain is characterized by two leucine heptad repeat motifs (LHR1 and LHR2), the VHID, PFYRE, and the SAW motifs. The N-terminal DELLA domain and C-terminal GRAS domain both play a role in binding DELLA proteins to be degraded by the ubiquitin ligase complex (Bolle, 2004; Dill et al. 2004; Murase et al., 2008) .

Mutations in and around the DELLA motif can dramatically alter plant growth and development. In wheat (*Triticum aestivum* L.), the *Reduced Height-1* (*Rht*) gene encodes the DELLA proteins. There is a single functional copy of *Rht* on each of the group four chromosomes (Ellis et al. 2002; Gale et al., 1975; McVittie et al., 1978; Sourdille et al., 1998). The two most well studied Rht alleles are the *Rht-B1b* and *Rht-D1b* semi-dwarfing mutations on the B and D genomes respectively (Allan, 1970; Allan et al., 1959; Gale & Gregory, 1977). These *Rht* mutations introduce a premature stop codon that partially suppresses GA response resulting in a semi-dwarf phenotype that is less susceptible to lodging and that can increase grain yield up to 20 % (Flintham et al., 1997; Hedden, 2003; Hoogendoorn et al., 1990; Pearce et al., 2011).

Genes belonging to the DELLA domain are present in many plant species. Characterized orthologs of the wheat *Rht* genes include *GA-Insensitive I* (*GAI*) and *Spindly* (*SPY*) in *Arabidopsis* (*Arabidopsis thaliana*); *Slender1* (*SLN1*) in barley (*Hordeum vulgare L.*) ; *Slender 1* (*SLR1*) in rice (*Oryza sativa L)* ; and *Dwarf 8* and *Dwarf 9* in maize (Brown et al., 2022; Ikeda et al. 2001; Wilhelm et al., 2013;P. M. Chandler et al., 2008; Chandler et al. 2002; Ikeda, Ueguchi-Tanaka et al., 2001; J. Peng et al., 1997; Winkler, 1994).

GA-response mutants that have been isolated from various plant species can either result in a phenotype characterized by being elongated, *slender*-mutants or, in a phenotype characterized by being GA-unresponsive, dwarf-mutants (Hooley, 1994; Swain & Olszewski, 1996). Mutations in *Rht* in wheat result in dwarf phenotypes but, in other homologous genes, mutations more likely result in elongated slender phenotypes (Ikeda et al. 2001). This phenomenon has been characterized in homologous genes of many plant species. In barley and rice the *Slender-1* mutation *SLN-1 (SLR-1 in rice)*, which is homozygous for the recessive allele is characterized by a rapid growth rate, slender appearance and limber plants that are unable to support themselves along with floral parts that are completely sterile (Foster, 1977). *Arabidopsis* contains two genes including *SPY,* which acts a regulator of the GA response, and *GAI,* which is a second signaling intermediate loss of function mutation, have also been shown to elongate stems and increase sterility (Jacobsen et al. 1996; Jacobsen & Olzewski, 1993; Kreppel, Blomberg, & Hart, 1997; Peng et al., 1997). *Rht* mutations that elicit a slender phenotype with sterile flower parts similar to those found in these homologous genes has not yet been characterized despite the characterization of several mutant *Rht* alleles including *Rht-B1b, Rht-D1b, Rht-B1c, Rht-B1d, Rht-B1e, Rht-D1c, Rht-D1d, Rht-8, Rht-3,* and *Rht0* (Flintham et al., 1997; Gale & Youssefian, 1985; Pearce et al., 2011).

Barley, rice and Arabidopsis are all diploid species so because they only contain one genome, the effects of mutations to essential genes needed for growth and development can lead to severe phenotypes. This is not the case in wheat because it is a hexaploid species consisting of an A, B, and D genome and there is a functional copy of *Rht* on each genome. This led us to hypothesize that varying doses of stop mutations in the *Rht-A1, Rht-B1,* and *Rht-D1* genes could lead to different dosage responses and the greater the dosage of *Rht* stop mutations the greater the effect would be on plant height in wheat. The dosage response would therefore create four different *Rht* genotype classes to test. Wheat plants containing stop mutations in each of the *Rht* genes would be the *Rht* triple mutant class and would likely exhibit the most severe height elongated phenotype. The *Rht* double mutant class would include plants containing stop mutations in any combination of two out of the three *Rht* genes and the *Rht* single mutant class would include plants that have a single *Rht* stop mutation in any of the three *Rht* genes. The last class is the *Rht* wild type class which contained no *Rht* stop mutations. The objectives of this study were to investigate the response to *Rht* stop mutation doses on plants grown under field conditions as well as upon coleoptile length in the presence and absence of GA.

Materials and Methods

Creation of Lines

The *Rht* nonsense mutations were induced by Jobson et. al (2019) using ethyl methanesulfonate (EMS) mutagenesis. The population was developed using Fortuna (CI 13596), a tall hard red spring wheat variety that carries no semi-dwarfing alleles (*Rht-A1a, Rht-B1a, Rht-D1a*). Lines were advanced to the $M_{1:3}$ generation. Nonsense mutations were found in each genome including the *Rht-A-Q555stop*, *Rht-B-Q420stop*, and the *Rht-D-W559stop*. *Rht-A-Q555stop* and *Rht-D-W559stop* are both positioned near the C terminus, while *Rht-B-Q420-stop* is more centrally located within the protein coding sequence. The *Rht* mutations were intercrossed to create homozygous wild-type and mutant classes at each of the three genomes to create eight genotype classes (Table 1.2). To reduce non-target mutations, all lines were then backcrossed to the non-mutagenized Fortuna twice and then advanced to create the BC_2F_2 populations (Jobson et al., 2020). Plants were allowed to self-pollinate in the greenhouse to create the $BCF_{2:3}$ population for use in field trials in Bozeman and Havre in 2021.

Genotypes at each generation were determined by collecting leaf tissue from individual two-week-old seedlings and performing DNA extraction using methods described by Reide & Anderson (1996). The DNA was used in PCR to amplify *Rht-A1, Rht-B1,* and *Rht-D1* using the nested PCR approach described by Li et al. (2013) which utilizes genome specific primers for initial *Rht* coding sequence amplification followed by non-genome specific *Rht* primers for secondary amplification. Following amplification of individual *Rht* segments, segments were sequenced by Sanger sequencing (GENEWIZ, Inc., Cambridge, MA). The *Rht* triple mutant class produced completely sterile heads. So to create *Rht* triple mutant population at each generation, plants that were heterozygous for the *Rht* in one genome but *Rht* mutant in two genomes (regardless of which Rht single gene was heterozygous) were planted and seedlings were genotyped for every experiment.

RNA Sequencing Expression Analysis

Data for RNA sequencing was acquired from three samples of Fortuna stem tissue and three samples of Fortuna leaf tissue previously collected and processed in Jobson et al. (2020) that were deposited in NCBI's Gene Expression Omnibus (Edgar et al. 2002) and accessible through GEO Series accession number GSE124940 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE124940). The sequence data were analyzed using ArrayStar (DNASTAR, Madison, Wisconsin), with the parameters set as: match setting at 100% for a minimum 370 bp, and all other settings left as default (Figure 2.2). The resultant data were presented as reads per kilobase of transcript for million mapped reads (RPKM) (Mortazavi et al. 2008) and normalized to *Triticum aestivum Ubiquitin*, a protein which was similarly expressed across genotypes, and previously reported as a reliably expressed gene for normalization (Tenea et al. 2011). *Rht* was analyzed against the available reference wheat genome and *Rht* expression levels were compared across each of the three wheat genomes.

Coleoptile Methods

To evaluate the dosage response of *Rht* stop mutations on coleoptile length and GA responsiveness, seeds were grown according to the cigar roll technique described by Bai et al. (2012). The seeds were surface-sterilized and then soaked in water for 24 h. Germination paper (Anchor Paper Co.) was soaked in water or in 100-µM GA_3 solution and 10 seeds from a single BC_2F_3 line were placed roughly 2 cm apart. Another presoaked germination paper was placed on top to sandwich the seeds in, and then rolled to a diameter of 3 cm. The randomized rolls were placed upright in racks within tubs that submerged the bottom 3 cm of the rolls in either dd H_2O or dd H_2O containing 100-µM GA_3 solution. BC_2F_3 lines from the Bozeman field trial in 2022 were used in these trials. There were five BC_2F_3 lines each with three biological replications, for each of the eight genotypes except for the *Rht* triple mutant class which had 30 lines homozygous for two *Rht* nonsense mutations and heterozygous for the third *Rht* nonsense mutation. Double *Rht* nonsense, single *Rht* heterozygotes were used because triple *Rht* nonsense mutant plants were invariably sterile. In total there were 150 rolls per replication with two replications. The tubs were placed in a dark room at 18 ˚C for 10 days. Coleoptile length was measured from the edge of the seed to the edge of the coleoptile tip in mm.

Field Methods

The effect of the *Rht* stop mutation doses were tested under field conditions. $BC_2F_{2:3}$ lines from each of the eight genotypes were planted in 2021 in a randomized complete block design with two replications. The number of lines varied for each genotype and are shown in Table 1.2 The trial was grown at the Northern Agricultural Research Center in Havre, Montana (48.52˚ N −109.76 ˚ W 804.7 m above sea level) on 20 April 2021 in Hilton clay loam (fine-loamy, mixed, active, mesic Oxyaquic Hapludalfs) with a pH 6.9. On 1 June, 0.4 l/ha of Vendetta (3,5-dibromo-4-Hydroxybenzonitrile 31.7%, 2-Ethylhexyl ester of 2-methyl-chlorophenoxyacetic acid 34% Wilbur-Ellis Co. Fresno, CA USA) and 0.4 1/ha of Affinity (Thifensulfuron-methyl 25%, Tribenuron-methyl 25% FMC Co. Philadelphia, PA USA) was applied for disease/weed control. The field was side banded with (100-20-10). A plot was a single 3-m row. Within each row, 20 seeds were sown by hand to a depth of 2.5 cm with 30-cm spacing between adjacent rows. This was a dryland site that received no additional moisture from irrigation. From 1 April to 31 August the station received 13.5 cm of precipitation. The highest recorded air temperature was 30.8 ˚C on 29 July 2021 and the lowest recorded air temperature was 0.2 ˚C on 21 April 2021 (https://agresearch.montana.edu/narc/weather_data/index.html).

The same trial was also planted at the Arthur H. Post Agronomy Farm in Bozeman, MT (45.67˚ N, 111.15˚ W, 1,455 m above sea level) in an Amsterdam silt loam (Fine-silty, mixed, superactive, frigid Typic Haplustolls) with a pH 6.5. Before planting, 289 kg/ha of Urea (46-0-0) was applied. The Bozeman trial was seeded according to the same specifications as the Havre planting listed above on 13 May 2021. From 1 May to 31 August, the research station received 17.2 cm of precipitation. Additionally, Five cm of irrigation was applied using hand line sprinklers on 22 June, 28 June, and 5 July. On 6 June, 1.75 l/ha of Vendetta (3,5-dibromo-4-Hydroxybenzonitrile 31.7%, 2-Ethylhexyl ester of 2-methyl-chlorophenoxyacetic acid 34% Wilbur-Ellis Co. Fresno, CA USA) and 0.77 l/ha of Parity (Fenoxaprop-P-Ethyl 11.3% Tenkoz Inc. Alpharetta, GA USA) were applied for disease/weed control. The highest recorded air temperature was 37.2 ˚C on 8 August 2021 and the lowest recorded the air temperature was -4.4 ˚C on 22 May 2021 (https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00241047/detail).

In 2022, the spaced plant trial was repeated and seeded according to the same seeding specifications as the previous year with three replications of a randomized complete block design. Before planting, 174 kg/ha of Urea (46-0-0) was applied. The trial was planted in Bozeman using BC_2F_3 lines derived from the Bozeman trial the previous year. The trial received five cm of irrigation was applied using hand line sprinklers on 21 June, 26 June, and 2 July.. From 1 May to 31 August, the research station received 19.8 cm of precipitation. The highest recorded air temperature was 33.8 ˚C on 1 August 2022 and the lowest recorded air temperature was -3.8 ˚C on 9 May 2022 (https://www.ncdc.noaa.gov/cdoweb/datasets/GHCND/stations/GHCND:USC00241047/detail). On 3 June, 0.05 l/ha of Affinity Tank Mix (Thifensulfuron-methyl 40%, Tribenuron methyl 10% E.I. DuPont de Nemours and Co. Wilmington, DE USA), 0.50 l/ha MCPE (2-methyl-4-chlorophenoxyacetic acid isocytl (2-ethylhexyl) ester 68.7%, Agrilliance, LLC. St. Paul MN USA), 1.17 l/ha of Discover (Clodinafop-propargyl 6.4%, Syngenta Crop Protection LLC. Greensboro, NC USA) were applied for weed and disease control. In both years, single plants were grown until maturity and then five representative plants from each plot were cut to ground level and bulk weighed and threshed.

Plant Measurements

All plant measurements were taken from five representative plants from each plot. Flag leaf length of a representative tiller was measured from culm to tip, and flag leaf width was measured from the widest point of the leaf 10 days post anthesis and all remaining field traits were measured at physiological maturity. Plant height was measured as the distance from the soil surface to the terminal spikelet of a representative tiller. Peduncle height was measured from the base of the topmost node to the terminal spikelet of a representative tiller. Productive tiller number was counted at the base of plants as only tillers with heads that set seed. Head height was measured from the terminal spikelet to the topmost spikelet. Plant weight was measured as biomass (included the weight of straw plus the weight of grain) and grain protein was analyzed with the LECO-FP 528 (LECO Co. St. Joseph, MI USA).

Statistical Analysis

Response variables from each field trial were analyzed via analysis of variance using a mixed linear model in R (R Foundation for Statistical Computing, Vienna, Austria). The model included block, genotype class and individual lines within each genotype class. and was implemented using the lme4 package in R. Individual lines within genotype classes were considered random and other factors as fixed effects using the lme4 package in R (Bates et al 2015). Statistical comparisons between the varying *Rht* genotype classes were made using a t statistic obtained from contrast statements using the emmeans package in R. Coleoptile length data were first analyzed with ANOVA for the water and GA treatments separately where the model was the same as for the field trials. Then the analysis was combined over the water and GA treatments, where the model included genotype class treatment (water or GA), genotype class x treatment, lines within genotype class, and lines within genotype class x treatment. Lines within genotype class and their interaction with treatment were considered random and other factors were considered fixed effects using the lme4 package in R (Bates et al 2015). Differences between water and GA for each genotype class were compared using a t statistic using contrast statements using the emmeans package. To compare differences in Rht expression levels among the three wheat genomes, two independent sample t-tests were used.

Results

2021 Field Trial

The effects of *Rht* stop mutation dosage between single, double, triple mutants and all wild-type plants were tested in 2021 in BC_2F_2 lines in spaced, single-plant trials in two locations. Due to varying environmental conditions between the 2021 environments, data are presented separately for each location. We made pairwise comparisons of the three genotypes listed in Table 1.2 that make up the double mutant class and single mutant class respectively and found no significant differences within classes so values are presented as averages for each class. The *Rht* triple mutant class exhibited an elongated, slender-type appearance (Figure 1.2) similar to the *SLN-1* mutation in barley, the *SLR-1* mutation in rice, and the *RGA* and *GA*I genes in *Arabidopsis* (Hooley, 1994, Swain and Olszeweski, 1996). In the Bozeman trial (Table 2.2) *Rht* triple mutants were on average 90.6 cm which was 14.4 cm (19%) taller (P<.0001) than the *Rht* all wild-type class, 9.9 (12.2 %) cm taller (P<.0001) than the *Rht* double mutant class, and 10.5 cm (13.2%) taller (P<.0001) than the *Rht* single mutant class. These same results were observed at the Havre location (Table 3.2), but the differences were compressed compared to Bozeman (Table 2.2). The addition of one and two mutants increased plant height 3.9 cm and 4.5 cm, respectively over the all wild type class. The Havre trial (Table 3.2) showed that the all-wild-type *Rht* plants did not significantly differ in plant height compared to the *Rht* single mutant class (51.2 vs. 53.5 cm) but the all-wild-type *Rht* class significantly differed from the *Rht* double mutants and were on average, 4.1 cm (7.5%) shorter (P<.0001). The *Rht* triple mutants had an average of 3.0 and 1.1 tillers per plant at Bozeman and Havre, respectively which was about ~15.5 (79.4%) and ~ 8.6 (87.1%) fewer tillers ($P<.0001$) than all other genotype classes at the two respective locations. The triple mutant class had on average of 9.9 spikelets which was ~5.7 (65%) fewer spikelets ($P<.0001$) than all other *Rht* genotype classes. All *Rht* triple mutant plants produced sterile heads and so yield traits were not considered. The *Rht* all-wild-type class did not significantly differ in any other field traits when compared to the single and double mutants in Bozeman or Havre. The *Rht* double mutants when compared to the *Rht* single mutants did not significantly differ in field or grain traits in either trial in 2021.

2022 Field Trial

In 2022, BC_2F_3 plants were grown in Bozeman in an irrigated spaced plant trial. The *Rht* triple mutant class again showed significant differences compared to every other dosage class (Table 4.2). *Rht* triple mutant plants were on average, 15.59 cm (21.3%) taller ($P<.0001$) than the *Rht* all-wild-type class, (20.7%) 15.22 cm taller ($P<.0001$) than the *Rht* single mutant class with and 13 cm (17.3%) taller ($P<.0001$) than the *Rht* double mutant class. The *Rht* triple mutant class also significantly differed ($P<.0001$) from every other genotype class in peduncle height, head height, leaf width and tiller number (Table 4.2).

The *Rht* single mutant class did significantly differ from the *Rht* double mutant class in full height and were on average 2.22 cm (6.3%) shorter ($P<0.05$) than the *Rht* double mutants. The *Rht* single mutants also had significantly shorter heads (2.9%) compared to the *Rht* double mutants ($P<0.05$). The *Rht* all-wild-type class did not significantly differ for any traits from the *Rht* single mutant or *Rht* double mutant class in 2022 but trended towards being shorter than the *Rht* double mutant class. We did not detect any significant grain trait differences in 2022 which are shown in Table 5.2 but with each single loss of an *Rht* mutation, grain weight decreased by approximately a gram. We also observed a similar trend in thousand kernel weight (Table 5.2).

Coleoptiles

When comparing the GA versus the H_2O treatment, the double *Rht* mutant class showed a significant increase ($P<0.05$) in coleoptile length in the presence of GA compared to water (Table 6.2). The *Rht*-all-wild type class showed the largest increase from GA (27.7 cm) (($P<0.0001$) compared to water. All other genotype classes did not significantly differ between GA and H_2O treatments.

The triple mutant class exhibited spindly, elongated coleoptiles similarly to the phenotype observed in field trials (Figure 1.2). It significantly increased length in both the GA and H_2O treatment. In the H_2O treatment, coleoptile length was increased by 63 mm (44.5%) ($P<.0001$) compared to the *Rht* all-wild type class while in the presence of GA, length was increased by 38.4 mm (35.9%) ($P<.0001$). Compared to the *Rht* single mutant class in the presence of GA, coleoptile length was increased 63.4 mm ($P<.0001$) and by 66.6 mm ($P<.0001$) in the presence of H_2O only. Compared to the *Rht* double mutant class in the GA treatment, *Rht* triple mutant coleoptile length was increased an average of 42.3 mm ($P<.0001$) and in the H_2O treatment was increased by 35.52 mm ($P<.0001$).

Coleoptile length was significantly increased by 28.26 mm in the *Rht* single mutant class compared to the wild-type class in the H_2O treatment ($P<.0001$) but was not significantly different in the presence of GA. However, when comparing the *Rht* double mutant class to the *Rht* all-wild-type class, coleoptile length was significantly increased in the *Rht* double mutant class by 20.6 mm but only when GA is present ($P<.0001$). Finally, the *Rht* double mutant class did significantly increase coleoptile length compared to the *Rht* single mutant class by 21.1 mm in the presence of GA ($P<.0001$) and by 31.1 mm in the H_2O only treatment ($P<0.0001$)

Expression Analysis of Leaf and Stem RNA

RNA sequencing data was analyzed for the *Rht* expression levels across each of the A, B and D genomes (Table 7.2). There was no significant difference in *Rht* expression levels in leaf tissue among any of the three genomes. However, in stem tissue, there was a significant increase in *Rht-B1* expression levels when compared to *Rht-D1* ($P<0.05$). There were no significant expression differences between the *Rht-A1* and *Rht-B1* or between *Rht-A1* and *Rht-D1* in stem tissue.

Discussion

Varying doses of *Rht* stop mutations impacted several field traits. In both field trials from 2021 and 2022, the *Rht* triple mutant class had significantly elongated plant height compared to every other genotype class (Figure 1.2). In Bozeman in 2021, *Rht* triple mutant plants were on average 19% taller than the *Rht* all-wild-type class, 13.2% taller than the *Rht* single mutant class and 12.2% taller than the *Rht* double mutant class (Table 2.2) . In 2022, we observed even greater height difference with *Rht* triple mutants on average that were 20 % taller than the *Rht* all-wild-type class, 15.2% taller than the *Rht* single mutant class and 17.3% taller than the *Rht* double mutant class (Table 4.2). *Rht* triple mutant plants all exhibited a severe phenotype with a spindly appearance and exaggerated bending at the nodes along with significantly longer peduncles and head lengths than all other *Rht* genotype classes. Furthermore, *Rht* triple mutant plants produced sterile heads. These observed characteristics are like those previously described from stop mutations in the orthologous genes from other plant species including *SLN1* in barley, *SLR1* in rice, and *RGA* and *GAI* in *Arabidopsis* (Foster, 1977; Jacobsen et al., 1996; Kreppel et al., 1997; Peng et al., 1997). These results demonstrate that the lack of at least one functional *Rht* gene results in severe defects to the plant. The presence of at least one functional *Rht* gene, regardless of which genome it was located on, resulted in normal, productive plants.

Plants containing two *Rht* double mutants were the second tallest class after the *Rht* triple mutant class. Having two *Rht* stop mutations significantly increased height when compared to the *Rht* all-wild-type class in the Bozeman trial and Havre trial in 2021 (Tables 2.2 and 3.2). In 2022, the *Rht* double mutant class was shown to significantly increase height and head length when compared to the *Rht* single mutant class but differ significantly from the *Rht* all-wild-type class (Table 4.2). Distinguishing dosage differences between the *Rht* all-wild-type class and the *Rht* single mutant class were variable between trial locations and years. In the 2021 Bozeman trial, the *Rht* single mutant class was significantly taller than the *Rht* all-wild-type class but in the Havre trial, there was no significant difference between the two classes (Tables 2.2 and 3.2). There was also no significant difference between the the single mutant class and all-wildtype class in 2022. These field results indicate that an increase in *Rht* stop mutations tends to increase plant height in a manner indicative of a dosage response however, as the amount of *Rht* stop mutations decreased it was more difficult to differentiate a height difference particularly between the *Rht* all-wild-type class and *Rht* single mutant class.

Aside from differences in full height and peduncle height, there were no differences found in other traits measured such as tillers, per plant flag leaf length and width, and yield traits from either 2021 or 2022 (Tables 2.2, 3.2 and 5.2). Likely even among the genotypes whose plant heights did differ significantly, the difference was not large enough to affect assimilate partitioning associated with changes in tillering or yield as in other mutations in *Rht* such as *Rht-B1b* and *Rht-D1b*.

Along with field trials a coleoptile length test was conducted to measure the impact of the *Rht* mutations on coleoptile length and responsiveness to GA (Table 6.2). The *Rht* triple mutant class has elongated, spindly coleoptiles that were significantly longer than all other *Rht* genotype classes regardless of whether GA was present or not. This finding is consistent with Lanahan and Ho (1988) who found that the *SLN-1* mutation in barley were not affected by GA treatments leading to the conclusion that phenotype of slender plants is independent of endogenous GA levels though this study used seedlings and measured full seedling height rather than coleoptile length only (Lanahan and Ho, 1988).

When comparing the GA to the H_2O coleoptile trials, there was a significant 10% increase in coleoptile length in the *Rht* double mutant class in the presence of GA. The *Rht*-all-wild-type class also increased by an average of 35% in the presence of GA which is consistent with previously reported findings in Jobson et al. (2020). When comparing *Rht* genotype classes in the H_2O treatment, after the *Rht* triple mutant class, the *Rht* double mutant class had the second longest coleoptiles that were on average 26.6% longer than the *Rht* single mutant class and 25.8% longer than the *Rht* all wild-type class. The *Rht* single mutant class and *Rht* all wild-type class did not differ in coleoptile length significantly. Similarly, to what was observed in the 2022 field trials a significant length the *Rht* single mutant class and *Rht* all wild-type class did not have a significant height difference.

RNA sequencing data was also analyzed to compare expression levels across *Rht-A1*, *Rht-B1* and *Rht-D1* (Table 7.2). Expression of *Rht* in leaf tissue was similar across all three genomes. Furthermore, in stems, there was no significant difference between *Rht-A1* and either *Rht-B1* or *Rht-D1*. However, we found that the *Rht-B1* had significantly higher expression than the *Rht-D1* in stems. We did not observe differences in the coleoptile or field experiments to suggest that a mutation in *Rht-B1* alone to have a greater height effect than mutations in *Rht-A1* or *Rht-D1* which is consistent with similar finding reported in Pearce et al. (2011). Even though there is a significant difference in expression between *Rht-B1* and *Rht-D1* it is likely not high enough to result in an observable difference phenotypically when either gene's function is lost.

Field experiments and coleoptile experiments showed that the lack of at least one *Rht* wild type gene (*Rht* triple mutant class) resulted in an elongated, spindly phenotype that was infertile (Figure 1.2). This is similar to the response exhibited previously for mutations in the genes orthologous to *Rht* in other plant species such as the *SLN* gene in barley, the *SLR1* gene in rice, and the *RGA* and *GAI* genes in *Arabidopsis* (Foster, 1977; Jacobsen et al., 1996; Kreppel et al., 1997; Peng et al., 1997). The addition of at least one *Rht* wild-type gene restores normal function to the plant allowing normal development and grain production. Differences in height and other traits among the *Rht* double mutant, *Rht* single mutants and *Rht* all wild-type dosages were more variable from trial to trial, though plants with higher doses of *Rht* mutants did trend towards increased height. Analyzing gene expression with RNA sequencing revealed that *Rht* gene is expressed at similar levels especially in leaf tissue while in stem tissue *Rht-B1* is expressed at higher levels compared to *Rht-D1*. These results help us to further characterize *Rht* function and its role in growth and development.

Table 1.2**:** depicts the genotypes of each *Rht* Dosage class and the number of lines for each genotype planted in the field.

Rht Class	***Rht*-Genotype**			**Number of lines in field**[a]
	A	**B**	**D**	
Triple Mutant Class	Rht-A1-555*	Rht-B1-420*	Rht-D1-559*	52
Double Mutant Class	Rht-A1-555*	Rht-B1-420*	Rht-D1a	32
	Rht-A1-555*	Rht-B1a	Rht-D1-559*	36
	Rht-A1a	Rht-B1-420*	Rht-D1-559*	35
Single Mutant Class	Rht-A1-555*	Rht-B1a	Rht-D1a	46
	Rht-A1a	Rht-B1-420*	Rht-D1a	46
	Rht-A1a	Rht-B1a	Rht-D1-559*	38
All-Wild-type Class	Rht-A1a	Rht-B1a	Rht-D1a	32

[a] Number BC_2F_2 lines in 2021 and BC_2F_3 lines in 2022

*indicates stop codon

Table 2.2 depicts mean field trait measurements taken from Bozeman in 2021.

***Rht* Dosage Classes and Comparisons**	**Full Height[a] cm**	**Primary Tillers[a] No./plant**	**Spikelets No./head[a]**	**Grain Weight[a] g/plant**	**Plant Weight[a] g/5 plants**	**Harvest Index[a]**	**TKW[b] milligram**
***Rht* Dosage Class Mean Values**							
Triple Mutant Class	90.6±1.0	2.95±1.02	9.48±0.32	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
Double Mutant Class	80.7±0.51	18.73±0.51	15.29±0.17	58.5±2.26	192.87±5.38	0.292±0.01	27.15±0.9
Single Mutant Class	80.1±0.58	19.36±0.58	15.37±0.17	59.1±2.24	196.61±5.37	0.295±0.01	28.03±0.5
All Wild-type Class	76.2±0.9	18.09±1	14.92±0.33	63.1±4.29	199.85±10.21	0.328±0.02	27.46±1.6
Contrast of *Rht* Dosage Class Values							
Double Mutant vs. Triple Mutant	9.86±1.13***	-15.78±1.14***	-5.8±0.37***	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
Single Mutant vs. Triple Mutant	10.53±1.13***	-16.41±1.4***	-5.89±0.37***	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
All-Wild-type vs. Triple Mutant	14.41±1.41***	-15.15±1.43***	-5.44±0.47***	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
Double Mutant vs. Single Mutant	-0.67±0.73	0.63±0.73	0.08±0.25	-2.12±0.85	-0.23±0.58	-0.208±0.23	0.58±1.41
All-Wild-type vs. Double Mutant	4.55±1.12***	0.63±1.12	0.37±0.39	4.14±1.27**	0.461±0.85	0.352±0.34	0.88±0.69
All-Wild-type vs. Single Mutant	3.88±1.12**	1.26±1.12	0.45±0.38	2.02±1.27	0.236±0.85	0.144±0.34	0.57±1.04

[a] Values represent the average for each *Rht* dosage class mean ± the standard error.

[b] Thousand Kernel Weight average for each *Rht* dosage class mean ± the standard error.

[c] Grain traits were not considered in the triple mutant class due to plant sterility.

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

Table 3.2 depicts mean field trait measurements taken from Havre in 2021

***Rht* Dosage Classes and Comparisons**	**Full Height[a] cm**	**Productive Tillers[a] No./plant**	**Spikelets No./head[a]**	**Grain Weight[a] g/5 plants**	**Plant Weight[a] g/5 plants**	**Harvest Index[a]**	**TKW[b] m/seed**
***Rht* Dosage Class Mean Values**							
Triple Mutant Class	65±1.05	1.14±0.77	9.91±0.23	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
Double Mutant Class	55.13±1.73	8.85±0.23	15.32±0.21	19.07±5.45	39.26±6.24	0.647±0.04	21.14±0.5
Single Mutant Class	52.9±2.01	8.61±0.4	15.07±0.17	16.29±4.56	38.75±8.31	0.595±0.01	22.14±0.508
All Wild-type Class	51.2±1.05	8.41±0.75	14.97±0.22	16.04±6.13	41.26±6.8	0.575±0.04	22.36±0.92
Contrast of *Rht* Dosage Class Values							
Double Mutant vs. Triple Mutant	9.6±1.23***	-7.62±0.84***	-5.48±0.33***	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
Single Mutant vs. Triple Mutant	11.72±1.23***	-7.39±0.84***	-5.28±0.33***	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
All-Wild-type vs. Triple Mutant	13.74±1.55***	-7.16±1.05***	-5.13±0.41***	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
Double Mutant vs. Single Mutant	-2.12±0.85**	-0.23±0.58	-0.21±0.23	-2.78±0.68	0.51±2.07	-0.052±0.03	1±0.71
All-Wild-type vs. Double Mutant	4.14±1.27**	0.46±0.85	0.35±0.34	3.03±0.89	-2.0±0.56	0.072±0.01	-0.22±1.05
All-Wild-type vs. Single Mutant	2.02±1.27	0.24±0.85	0.14±0.34	0.25±1.57	2.51±2.07	0.02±0.03	-1.23±1.04

[a] Values represent the average for each *Rht*dosage class mean ± the standard error.

[b] Thousand Kernel Weight average for each *Rht*dosage class mean ± the standard error.

[c] Grain traits were not considered in the triple mutant class due to plant sterility.

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

Table 4.2 depicts mean field trait measurements taken from Bozeman in 2022.

Rht- 1 **Dosage Classes and Comparisons**	**Full Height[a] cm**	**Peduncle Height[a] Cm**	**Head Height[a] cm**	**Leaf Length[a] cm**	**Leaf Width[a] cm**	**Primary Tillers[a] No./plant**
Rht- 1 **Dosage Class Mean Values**						
Triple Mutant Class	88.7±1.69	41.32±0.55	9.31±0.13	17.14±2.77	0.66±0.04	3.58±0.5
Double Mutant Class	75.7±0.75	35.01±0.24	8.4±0.05	19.33±1.19	1.31±0.02	14.53±0.22
Single Mutant Class	73.5±0.74	34.39±0.24	8.17±0.05	16.61±1.19	1.32±0.02	14.54±0.21
All Wild-type Class	73.2±1.41	34.2±0.46	8.18±0.1	16.83±2.26	1.32±0.03	14.64±0.41
Contrast of *Rht* Dosage Class Values						
Double Mutant vs. Triple Mutant	13±1.85***	6.31±0.6***	0.91±0.14***	-2.19±3.01	-0.65±0.04***	-10.95±0.54***
Single Mutant vs. Triple Mutant	15.22±1.85***	6.93±0.59***	1.14±0.14***	0.54±3.01	-0.66±0.04***	-10.96±0.54***
All-Wild-type vs. Triple Mutant	15.59±2.2***	7.12±0.71***	1.14±0.16***	0.31±3.57	-0.67±0.05***	-11.05±0.64***
Double Mutant vs. Single Mutant	-2.223±1.05*	-0.62±0.34	-0.23±0.08*	-2.73±1.68	0.007±0.02	0.02±0.3
All-Wild-type vs. Double Mutant	2.59±1.59	0.82±0.52	0.24±0.12*	2.5±2.55	-0.01±0.03	-0.11±0.03
All-Wild-type vs. Single Mutant	0.36±1.59	0.19±0.51	-0.003±0.12	-0.23±2.55	-0.004±0.03	-0.1±0.46

[a] Values represent the average for each genotype ± the standard error.

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

Table 5.2 depicts mean grain trait measurements taken from Bozeman in 2022.

***Rht* Dosage Classes and Comparisons**	**Grain Weight g/ 5 plants**	**Plant Weight g/ 5 plants**	**TKW[b] mg/seed**	**Harvest Index**	**Protein Content %**
***Rht* Dosage Class Mean Values**					
Triple Mutant Class	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
Double Mutant Class	56.9±2.98	149.2±3.42	31.9±0.04	0.377±0.03	16.9±0.27
Single Mutant Class	58.7±2.96	149.1±3.4	30.1±0.21	0.391±0.02	16.8±0.27
All Wild-type Class	59.5±5.28	149.4±6.44	32.5±0.73	0.394±0.06	16.6±0.6
Contrast of *Rht* Dosage Class Values					
Double Mutant vs. Triple Mutant	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
Single Mutant vs. Triple Mutant	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
All-Wild-type vs. Triple Mutant	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]	*NA*[c]
Double Mutant vs. Single Mutant	1.79±4.2	-0.14±4.82	1.789±4.2	0.014±0.04	-0.04±0.39
All-Wild-type vs. Double Mutant	-0.75±6.36	-0.31±7.28	-2.54±6.37	-0.003±0.96	0.25±0.59
All-Wild-type vs. Single Mutant	-2.54±6.37	-0.18±7.29	-0.751±6.36	-0.02±0.06	0.29±0.59

[a] Values represent the average for each *Rht* dosage class mean ± the standard error.

[b] Thousand Kernel Weight average for each *Rht* dosage class mean ± the standard error.

[c] Grain traits were not considered in the triple mutant class due to plant sterility.

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

Table 6.2 depicts the mean coleoptile length among each *Rht* dosage class in the presence of GA and in H_2O.

***Rht* Genotype**	**Coleoptile Length H_2O only mm**	**Coleoptile Length GA mm**	**Contrast of Treatment Values**
	***Rht* Dosage Class Mean Values**		**H2O vs. GA**
Triple Mutant Class	145.8±3.34	142±3.06	3.8±5.8
Double Mutant Class	110.23±2.97	100.46±2.87	9.83±4.1**
Single Mutant Class	79.21±3.48	79.43±2.85	-0.2±4.9
All Wild-type Class	107.5±2.79	79.8±2.8	27.7±3.9***
Contrast of *Rht* Dosage Class Values			
Double Mutant vs. Triple Mutant	35.52±3.86***	42.3±3.41***	
Single Mutant vs. Triple Mutant	66.62±4.05***	63.4±3.41***	
All-Wild-type vs. Triple Mutant	38.36±4.6***	63±3.90***	
Double Mutant vs. Single Mutant	-31.1±3***	-21.1±1.09***	
All-Wild-type vs. Double Mutant	2.84±3.7	20.6±2.7***	
All-Wild-type vs. Single Mutant	-28.26±3.9***	-0.4±2.8	

[a] Values represent the average for each *Rht* dosage class mean ± the standard error.

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

Table 7.2 Comparison of *Rht* expression values in leaves and stems. Data represents the average ± the standard error and is reported as the reads per kilobase million (RPKM), n=3,

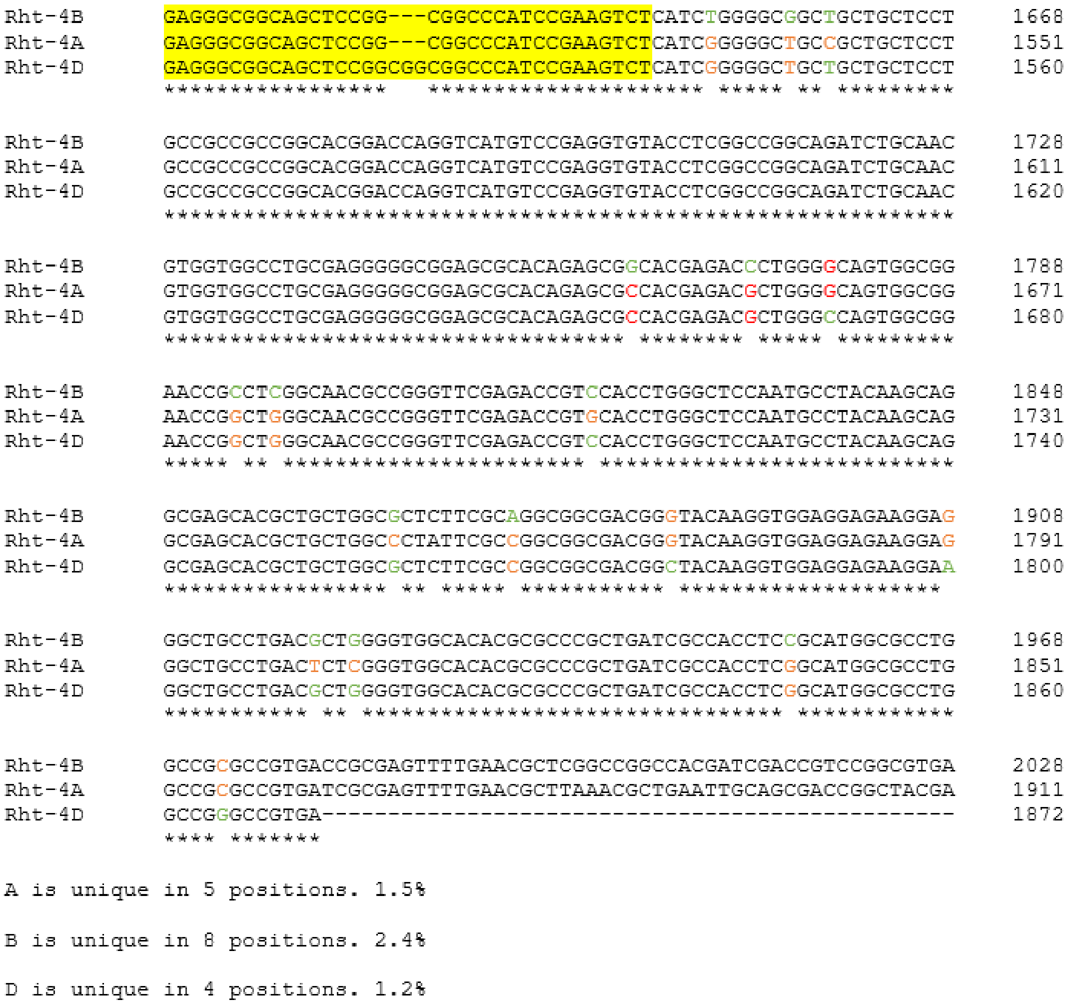
Gene Name	**GenBank Accession Number**	**Stems Average RPKM**	**Leaves Average RPKM**
***Rht* Genes**			
Rht-A1	FR668584	18618.81±18618.8	7724.7±1485.9
Rht-B1	FR668586	21182.11±1534	10345.4±1403.2
Rht-D1	AJ242531	13043.56±1623.3	7005.7±761.2
Housekeeping Gene			
Ubiq		2091102	2266657
Genome Comparisons			
A vs B		-2563.3±684.5	-2620.7±82.7
A vs D		5575.23±595.2	719±724.7
B vs D		8138.6±89.3*	3339.7±642

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

Figure 1.2 depicts *Rht*-triple-stop mutation and *Rht* double-stop mutation plants at full maturity

Rht **Triple-stop mutant** ***Rht*** **Double-stop mutant**

Figure 2.2: The last ~370 bp of Rht alignment in ‘Fortuna’. 335bp RNA-Seq started after highlighted region. Mer at 20 set to 100%



```
Rht-4B      GAGGGCGGCAGCTCCGG---CGGCCCATCCGAAGTCTCATCTGGGGCGGCTGCTGCTCCT    1668
Rht-4A      GAGGGCGGCAGCTCCGG---CGGCCCATCCGAAGTCTCATCGGGGGCTGCCGCTGCTCCT    1551
Rht-4D      GAGGGCGGCAGCTCCGGCGGCGGCCCATCCGAAGTCTCATCGGGGGCTGCTGCTGCTCCT    1560
            *****************   ********************* ***** ** *********

Rht-4B      GCCGCCGCCGGCACGGACCAGGTCATGTCCGAGGTGTACCTCGGCCGGCAGATCTGCAAC    1728
Rht-4A      GCCGCCGCCGGCACGGACCAGGTCATGTCCGAGGTGTACCTCGGCCGGCAGATCTGCAAC    1611
Rht-4D      GCCGCCGCCGGCACGGACCAGGTCATGTCCGAGGTGTACCTCGGCCGGCAGATCTGCAAC    1620
            ************************************************************

Rht-4B      GTGGTGGCCTGCGAGGGGGCGGAGCGCACAGAGCGGCACGAGACCCTGGGGCAGTGGCGG    1788
Rht-4A      GTGGTGGCCTGCGAGGGGGCGGAGCGCACAGAGCGCCACGAGACGCTGGGGCAGTGGCGG    1671
Rht-4D      GTGGTGGCCTGCGAGGGGGCGGAGCGCACAGAGCGCCACGAGACGCTGGGCCAGTGGCGG    1680
            *********************************** ******** ***** *********

Rht-4B      AACCGCCTCGGCAACGCCGGGTTCGAGACCGTCCACCTGGGCTCCAATGCCTACAAGCAG    1848
Rht-4A      AACCGGCTGGGCAACGCCGGGTTCGAGACCGTGCACCTGGGCTCCAATGCCTACAAGCAG    1731
Rht-4D      AACCGGCTGGGCAACGCCGGGTTCGAGACCGTCCACCTGGGCTCCAATGCCTACAAGCAG    1740
            ***** ** *********************** ***************************

Rht-4B      GCGAGCACGCTGCTGGCGCTCTTCGCAGGCGGCGACGGGTACAAGGTGGAGGAGAAGGAG    1908
Rht-4A      GCGAGCACGCTGCTGGCCCTATTCGCCGGCGGCGACGGGTACAAGGTGGAGGAGAAGGAG    1791
Rht-4D      GCGAGCACGCTGCTGGCGCTCTTCGCCGGCGGCGACGGCTACAAGGTGGAGGAGAAGGAA    1800
            ***************** ** ***** *********** ******************** 

Rht-4B      GGCTGCCTGACGCTGGGGTGGCACACGCGCCCGCTGATCGCCACCTCCGCATGGCGCCTG    1968
Rht-4A      GGCTGCCTGACTCTCGGGTGGCACACGCGCCCGCTGATCGCCACCTCGGCATGGCGCCTG    1851
Rht-4D      GGCTGCCTGACGCTGGGGTGGCACACGCGCCCGCTGATCGCCACCTCGGCATGGCGCCTG    1860
            *********** ** ******************************** ************

Rht-4B      GCCGCGCCGTGACCGCGAGTTTTGAACGCTCGGCCGGCCACGATCGACCGTCCGGCGTGA    2028
Rht-4A      GCCGCGCCGTGATCGCGAGTTTTGAACGCTTAAACGCTGAATTGCAGCGACCGGCTACGA    1911
Rht-4D      GCCGGGCCGTGA------------------------------------------------    1872
            **** *******

A is unique in 5 positions. 1.5%

B is unique in 8 positions. 2.4%

D is unique in 4 positions. 1.2%
```

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CHAPTER THREE:

GENERATING AND TESTING RHT-A1 MUTATIONS IN SPRING WHEAT

Introduction

The *Reduced Height* (*Rht*) gene plays a critical role in regulating plant growth and development by encoding DELLA (Asp-Glu-Leu-Leu-Ala) proteins that negatively regulate the plant's response to gibberellic acid (GA) (Zentella et al. 2007.). In wheat (*Triticum aestivum L.*), the *Rht* gene is present on each of three genomes, A, B and D, on the group four chromosomes and certain mutations in these genes have been shown to affect DELLA protein's ability to respond to GA resulting in a semi-dwarf phenotype. The *Rht-B1b* and *Rht-D1b* mutations on the B and D genomes respectively, were introduced into wheat breeding programs in the 1960's and 1970's (Hedden, 2003). Both *Rht-B1b* and *Rht-D1b* contain a premature stop codon near the *Rht* N-terminus that partially represses GA response. (Peng et al., 1999). These mutations have been well-studied and extensively incorporated into standard-height varieties via crossing to create semi-dwarf wheat varieties that are resistant to lodging and can increase grain yield up to 21% (Flintham et al. 1997; Hedden, 2003; Hoogendoorn et al. 1990). A study performed by Jobson et al (2018) even found that *Rht-B1b/Rht-D1b* positively impacted flour yield and dough mixing strength when analyzing baking quality. Despite these benefits, *Rht-B1b* and *Rht-D1b* are also associated with pleiotropic effects including decreased seed size and protein content (Appleford et al. 2006; Casebow et al., 2016; Gale & Youssefian, 1985). In certain environments, *Rht-B1b* and *Rht-D1b* mutations can lead to decreased emergence due to shorter coleoptile lengths and decreased seedling leaf area; especially in higher drought areas (Allan, 1970). *Rht-D1b* has even been found to increase susceptibility to *Fusarium* head blight by interfering with type 1 resistance which is the resistance that can prevent initial infection (Srinivasachary et al., 2008). The use of alternative *Rht* alleles that impart a semi-dwarf phenotype upon wheat plants while carrying less adverse effects would be useful in many environments.

Since the 1960s many other allelic variants of *Rht* have been characterized. However, none of these alleles have been as widely incorporated into wheat varieties as *Rht-B1b* or *Rht-D1b*. No *Rht-A1* mutations have been found that decrease height to a beneficial degree or increase yield traits. Newly discovered *Rht* alleles could be used to achieve a more diverse range of plant height and agronomic phenotypes. The presence of *Rht-B1b* or *Rht-D1b* alone reduces plant height by 14.6%, but the presence of *Rht-B1b* and *Rht-D1b* together reduces height by 41% (Flintham et al., 1997).

Our objective here was to cross *Rht-A1* mutations into adapted varieties and measure the effects of these mutations both on their own and in conjunction with *Rht-B1b* in a spaced planted field trial. We tested the eight *Rht-A1* missense and one nonsense mutations listed in Table 1.3 These mutations were previously included in a yeast-two hybrid assay to test Rht-A1 interaction with GID1 both in the presence and absence of GA (Brown et al., 2022). Brown et. al (2022) showed that the *Rht-A1-Q6** nonsense allele did not interact with GID1 in the presence or absence of GA, identical to the pattern observed with Rht-B1b, which indicated that this mutation may have the greatest effect on plant traits. The lack of interaction was previously demonstrated of Rht-B1b with GID1 was previously demonstrated (Brown et al., 2022; Pearce et al. 2011). The eight Rht-A1 missense mutations varied in their degree of GID1 binding. *Rht-A1-*

S50F, *Rht-A1-P94L*, *Rht-A1- V55M*, and *Rht-A1-E63K* had no GID1 interaction in the absence of GA, but their function was fully restored to *Rht-A1* levels when GA was present. *Rht-A1-L358F* and *Rht-A1-A86V* interacted slightly with GID1 in the absence of GA. *Rht-A1-A53V* and *Rht-A1-R3C* showed an intermediate interaction between the positive and negative controls. In the presence of GA, *Rht-A1-L358F, Rht-A1-A86V, Rht-A1-A53V* and *Rht-A1-R3C* all bound to GID1. *Rht-A1-A53T* appeared to have the same degree of binding to GID1 in the presence and absence of GA, but the interpretation of results for this mutation could be unreliable. Brown et al (2022) indicated alleles that grew on interacted with GID1 in the presence and absence of GA in a similar manner to wild type alleles are expected to exhibit plant growth similar to wild-type plants, whereas alleles that differed when GA was or was not present are expected to produce plants that have significant height differences.

Based on these results we expected plants carrying *Rht-A1-Q6** to have the greatest height reduction as *RhtT-A1-Q6** behaved like *Rht-B1b* and *Rht-D1b* in not binding to GID1 in yeast-two hybrid assays. Many of the missense mutations tested in the assay did have different degrees of GID1 binding in the absence of GA. Therefore, testing in vivo is required to determine which alleles impact wheat plant height.

Methods

Creation of Rht-A1 Mutant Alleles

Ten thousand seeds of the soft white spring variety Alpowa (PI 566596) were mutagenized using EMS mutagenesis using the method described by Slade et. al (2005) and modified by Feiz et al, (2009). The population was advanced through single seed decent to the M_2 generation. Two thousand M_2 lines were screened for mutations in the *Rht-A1* gene. For screening, DNA was extracted from leaf tissue from a bulk of three individual M_3 plants for each of 2,000 M_2 lines. Amplification of *Rht-A1* was done using the nested PCR approach described by Li et al. (2013) in which genome specific primers are first used for initial amplification and then non-genome specific *Rht* primers are used for secondary amplification. Following amplification of individual *Rht* segments, segments were sequenced by Sanger sequencing (GENEWIZ, Inc., Cambridge, MA). Eight missense and one nonsense *Rht* mutation were chosen based on SIFT (sorting intolerant from tolerant) prediction software to estimate whether the EMS induced single nucleotide polymorphisms would have a neutral or deleterious impact on Rht function (Table 1.3). Each of the 9 *Rht-A1* mutations was crossed to the non-mutagenized, semi-dwarf hard red spring parent Duclair (PI 660981), which is a Montana-adapted variety to introduce the *Rht-B1b* allele to the F_1 population.

Creation of BC_4F_2 and BC_5F_2 Lines

Along with Duclair, Vida (PI 642366) was also used as a recurrent parent for backcrossing and is another Montana-adapted semi-dwarf hard red spring variety. Backcrosses to Duclair continued to the BC_5 generation and backcrosses to Vida continued to the BC_4 generation. After each round of backcrossing, plants were genotyped by sequencing for *Rht-A1* alleles and for *Rht-B1b*. Only plants heterozygous for *Rht-B1b* and *Rht-A1* were kept for advancement. We finished with a total of 16 BC_4F_1 or BC_5F_1 lines which were then allowed to self-pollinate to create BC_4F_2 and BC_5F_2 lines in Vida/Duclair and Duclair respectively.

Field Trials 2022

A spaced-plant field experiment was planted on May 18, 2022 at the Arthur H. Post Agronomy Farm in Bozeman, MT (45.67˚ N, 111.15˚ W, 1, 1455 m above sea level) in an

Amsterdam silt loam (Fine-silty, mixed, superactive, frigid Typic Haplustolls) with a pH of 6.5. Before planting, 174 kg/ha of Urea (46-0-0) was applied. Two hundred BC_4 or BC_5F_2 seeds from each of the 16 crosses were hand-planted 15 cm apart within a row. Twenty seeds were planted to a depth of 2.5 cm per 3 m row with 30-cm spacing between adjacent rows. A plot consisted of 10 rows each 3-m long. On 3 June, 0.05 l/ha of Affinity Tank Mix (Thifensulfuron-methyl 40%, Tribenuron methyl 10% E.I. DuPont de Nemours and Co. Wilmington, DE USA), 0.50 l/ha MCPE (2-methyl-4-chlorophenoxyacetic acid isocytl (2-ethylhexyl) ester 68.7%, Agrilliance, LLC. St. Paul MN USA), 1.17 l/ha of Discover (Clodinafop-propargyl 6.4%, Syngenta Crop Protection LLC. Greensboro, NC USA) were applied for weed and disease control. Hand line sprinklers were used to apply 5 cm of irrigation on 21 June, 26 June, and 2 July. From 1 May to 31 August, the research station received 19.8 cm of precipitation. The highest recorded air temperature was 33.8 ˚C on 1 August 2022 and the lowest recorded air temperature was -3.8 ˚C on 9 May 2022 (https://www.ncdc.noaa.gov/cdoweb/datasets/GHCND/stations/GHCND:USC00241047/detail).

Leaf tissue from each plant was collected at the four-leaf-stage and DNA was extracted for sequencing to determine the *Rht-A1* mutant and *Rht-B1b* genotypes using the protocols described previously (Li et al 2013). Only *Rht* homozygous plants were considered in the trial and these plants were tagged for measurement and harvest.

Measurements

At physiological maturity, plant height was measured from the soil surface to the base of the terminal spikelet from a representative tiller of each plant. Single plants were then harvested and threshed using a single plant thresher. Yield was measured as the total grain produced from each plant and grain weight was calculated after counting out and weighing a sample of 200 seeds using a seed counter.

Statistical Analysis

Comparisons between mutant and wild-type means in each cross were made using two-tailed, independent sample t-tests.

Results

Our initial field trial indicates that some of the novel *Rht-A* mutations did influence agronomic traits and are shown in Tables 2.3 and 3.3. The *Rht-A1-E63K* mutation had the greatest impact on agronomic traits. All *Rht-A1-E63K* crosses in both the standard-height and semi-dwarf backgrounds showed a trend of decreasing height. Though the *Rht-A1-E63K* mutation in the standard-height Duclair background was the only cross that showed a significant height reduction by 4.22% compared to the wild-type (P=0.01). There also appeared to be a significant reduction in grain yield with the *Rht-A1-E63K* allele by 10.9% in the semi-dwarf Duclair background (P<.0001). In the standard-height Vida background the *Rht-A1-E63K* mutant significantly increased thousand kernel weight by 2.9% (P=0.05) (Table 3.3).

The *Rht-A1-Q6** allele also impacted agronomic traits. The *Rht-A1-Q6** allele significantly decreased thousand kernel weight in the semi-dwarf Vida background by 4.9% (P<.0001). Furthermore, though there was no significant difference among heights, the standard-height background of both the Vida and Duclair crosses trended towards decreased height with the *Rht-A1-Q6** mutation.

The *Rht-A1-V55M* allele significantly decreased height by 4.4% compared to the wild type but only in the semi-dwarf Vida background (P value= 0.01). The *Rht-A1-53T* allele appeared to increase grain weight by 10.2% in the semi-dwarf Duclair background (P=0.02). There was also a thousand-kernel weight increase trend in the standard-height Duclair background with *Rht-A1-53T* allele and trended towards an increase in grain yield compared to the wild-type in both backgrounds as well. *Rht-A1-R3C* did not significantly affect any measured traits but did consistently trend towards increasing height by an average of 2 cm in each background. We did not detect any differences in allelic class means in any other traits.

Discussion

Nine *Rht-A*1 EMS induced mutations were backcrossed to two hard red spring varieties and tested in a spaced-plant field trial to test height and grain trait effects. NILs were created by crossing each of the nine mutations into two Montana-adapted parent lines and 16 of these mutant genotypes were compared to their corresponding wild-type sister line. Based on our observations *Rht-A1-E63K*, *Rht-A1-Q6**, *Rht-A1-V55M*, and *Rht-A1-53T* all had either significantly changed height or grain traits.

Rht-A1-E63K impacted the most height and grain traits. It decreased plant height in the Duclair background in the presence of *Rht-B1b* (72 cm vs 75 cm). The *Rht-A1-E63K* mutation also showed strong trends of decreasing height in both the semi-dwarf and standard height backgrounds. In the standard height Vida background, *Rht-A1-E63K* increased grain weight while significantly decreasing total grain yield in the semi-dwarf Duclair background (14 vs. 15.8 g/plant).

We expected *Rht-A1-Q6** nonsense allele to have the highest impact on agronomic traits but we observed no significant differences in plant height compared to the wild-type. We also observed the *Rht-A1-Q6** nonsense allele significantly decreased total grain yield in the Vida background in the presence of *Rht-B1b*. These results do not match what we expected to see based on the findings from Brown et al (2022) in which the yeast-two hybrid assay demonstrated that *Rht-A1-Q6** had very little interaction with GID1 regardless of whether GA was present. This interaction of *Rht-A1-Q6** was similar to how *Rht-B1b* interacts to GID1 indicating that *Rht-A1-Q6** would likely affect plant height. Since our trial was grown in spaced plant conditions, this could have reduced resource competition between lines, which could have prevented further observed differences in plant height and grain traits. Furthermore, Bozeman receives higher precipitation rates than most of Montana's wheat growing regions.

It was observed that *Rht-A1-V55M* significantly decreased plant height but only in the presence of *Rht-B1b* in the Vida cross (70.5 cm vs 73.8 cm) and out of all the alleles we tested this was the only allele that decreased height without decreasing grain yield or thousand kernel weight. Lastly, *Rht-A1-53T* did not have any effect on plant height but did significantly increase thousand kernel weight in the Duclair cross in the presence of *Rht-B1b* (31vs 28mg).

Currently, no height-reducing *Rht-A1* alleles have been identified in hexaploid wheat. (Flintham et al., 1997; Gale, Law, & Worland, 1975; Gale & Youssefian, 1985; Pearce et al., 2011). Brown et al. (2022) did discover two *Rht-A1* missense mutations that significantly reduced plant height but only in durum wheat. Our results suggest that when crossed into adapted hexaploid varieties, *Rht-A1* mutations have the potential to affect plant height. Though none of the *Rht-A1* alleles affected plant height as much as *Rht-B1b*, we did still observe significant differences. The *Rht-A1-E63K* mutation and *Rht-A1-V55M* mutation both significantly decreased plant height when compared to the wild-type. There is likely no agronomic benefit to further reducing height using *Rht-A1* mutations if a plant already carries *Rht-B1b*. However, we did observe some mutations to trend towards slightly increasing height in the presence of *Rht-B1b* such as the *Rht-A1-R3C* mutation which could potentially be beneficial if a slight height increase could alleviate some of the pleiotropic affects that *Rht-B1b* has such as increasing coleoptile length or protein content.

We also observed mutations that caused a significant difference in grain characteristics. Some of these changes in grain traits did negatively impact grain yield when compared to their wild-type sister lines. We also observed that *Rht-A1-53T* and *Rht-A1-E63K* increased grain size though full density trials will be needed to confirm grain impacts.

Further research will be necessary to fully characterize the agronomic impact of the novel *Rht-A1* alleles. The results of this study are limiting as the trial we performed consisted only of spaced plant trials in which conditions are different than those at normal planting density. The study also took place in only one environment during one season. Because of this, further trials across multiple locations and years should be conducted to fully determine the impacts of these novel *Rht-A* mutations on important agronomic traits. In addition, experiments to determine the impact of these *Rht-A* mutations on coleoptile length should be conducted.

Ultimately, studying these *Rht-A1* mutations can aid in our understanding of how *Rht-A1* impacts plant growth and development. The *Rht-A1* alleles reported here could also improve genetic diversity that leads to the development of new cultivars.

Table 1.3: Summary of Rht-A mutations including location and predicted effect on protein function

MALP[a] ID M_2	Allele	Base Pair #	Amino Acid #	Orig. Codon	New Codon	Original Amino Acid	New Amino Acid	SIFT[b]	Effect
1536	Rht-A1-R3C	7	3	cgc	tgc	R	C	-4.9	Deleterious
1916	Rht-A1-R3H	8	3	cgc	cac	R	H	-2.77	Deleterious
1158	Rht-A1-Q6*	16	6	cag	tag	Q	Stop	NA	Deleterious
2141	Rht-A1-A53T	157	53	gcg	acg	A	T	-2.73	Deleterious
1930	Rht-A1-A53V	158	53	gcg	gtg	A	V	-2.74	Deleterious
1487	Rht-A1-V55M	163	55	gtg	atg	V	M	-2.32	Neutral
1461	Rht-A1-E63K	187	63	gag	aag	E	K	-3.28	Deleterious
1475	Rht-A1-A86V	257	86	gcc	gtc	A	V	-2.31	Neutral
1305	Rht-A1-P94L	281	94	ccc	ctc	P	L	-8.81	Deleterious

[a] MALP short for mutagenized Alpowa

[b] SIFT; sorting intolerant from tolerant, more deleterious mutations have values closer to 0 with values less than 0.05 predicted to be deleterious to protein function.

Table 2.3: Impact of Rht-A mutations on genotype class mean values in Vida for agronomic traits in Bozeman 2022 field trials.

Cross[a]	Rht-A Genotype	Rht-B Genotype	n[b]	Plant Height[c] cm	Total Grain Yield[c] g/plant	Thousand Kernel Weight[c] mg/seed
Vida*4//Duclair/MALP 1158	Rht-A1-Q6	Rht-B1b	11	71.91±1.74	14.85±1.79***	29.89±0.38
Vida*4//Duclair/MALP 1158	Rht-A1a	Rht-B1b	10	72.30±0.87	16.04±1.71	31.44±0.62
Vida*4//Duclair/MALP 1158	Rht-A1-Q6	Rht-B1a	4	83.25±2.14	16.35±2	32.47±0.34
Vida*4//Duclair/MALP 1158	Rht-A1a	Rht-B1a	12	85.83±0.72	15.2±1.38	33.2±0.86
Vida*4//Duclair/MALP 1305	Rht-A1-P94L	Rht-B1b	12	68.25±0.75	19.12±1.84	31.67±0.19
Vida*4//Duclair/MALP 1305	Rht-A1a	Rht-B1b	13	66.23±1.14	16.06±1.76	34.14±0.75
Vida*4//Duclair/MALP 1305	Rht-A1-P94L	Rht-B1a	10	80.20±1.16	20.1±2.14	33.13±0.53
Vida*4//Duclair/MALP 1305	Rht-A1a	Rht-B1a	16	79.56±0.89	18.43±1.4	34.91±0.34
Vida*4//Duclair/MALP 1461	Rht-A1-E63K	Rht-B1b	12	70.42±1.29	13.94±1.92	33.86±1.46
Vida*4//Duclair/MALP 1461	Rht-A1a	Rht-B1b	11	72.0±1.46	13.83±0.95	28.03±0.63
Vida*4//Duclair/MALP 1461	Rht-A1-E63K	Rht-B1a	9	84.33±1.75	15.14±2.52	30.26±1.01*
Vida*4//Duclair/MALP 1461	Rht-A1a	Rht-B1a	11	87.36±1.71	15.07±1.26	29.37±0.41
Vida*4//Duclair/MALP 1475	Rht-A1-A86V	Rht-B1b	11	67.27±0.85	14.54±1.46*	31.71±0.74
Vida*4//Duclair/MALP 1475	Rht-A1a	Rht-B1b	12	67.33±1.08	20.43±2.13	31.69±0.48
Vida*4//Duclair/MALP 1475	Rht-A1-A86V	Rht-B1a	9	79.22±1.85	14.27±6.77	32.69±2.04
Vida*4//Duclair/MALP 1475	Rht-A1a	Rht-B1a	11	81.55±0.92	16.17±1.37	31.94±1.22
Vida*4//Duclair/MALP 1487	Rht-A1-V55M	Rht-B1b	16	70.50±0.7**	15.18±0.76	33.52±0.64
Vida*4//Duclair/MALP 1487	Rht-A1a	Rht-B1b	9	73.78±0.97	16.1±2.67	33.19±0.97
Vida*4//Duclair/MALP 1487	Rht-A1-V55M	Rht-B1a	13	82.15±1.88	16.96±2.33	31.95±1.17
Vida*4//Duclair/MALP 1487	Rht-A1a	Rht-B1a	8	83.88±1.27	17.09±0.93	31.95±0.62
Vida*4//Duclair/MALP 1536	Rht-A1-R3C	Rht-B1b	8	70.13±0.58	13.07±1.8	32.08±0.44
Vida*4//Duclair/MALP 1536	Rht-A1a	Rht-B1b	9	68.44±1.51	15.03±2.64	31.95±0.65
Vida*4//Duclair/MALP 1536	Rht-A1-R3C	Rht-B1a	6	80.33±1.17	14.15±2.06	34.51±1.85
Vida*4//Duclair/MALP 1536	Rht-A1a	Rht-B1a	5	78.6±1.36	16.6±2.3	32.72±1.18
Vida*4//Duclair/MALP 1916	Rht-A1-R3H	Rht-B1b	11	66.82±1.34	17.63±1.43	33.62±2.96
Vida*4//Duclair/MALP 1916	Rht-A1a	Rht-B1b	14	68.93±0.74	19.19±1.25	31.25±0.48
Vida*4//Duclair/MALP 1916	Rht-A1-R3H	Rht-B1a	5	76.6±2.14	20.96±1.48	30.99±0.61
Vida*4//Duclair/MALP 1916	Rht-A1a	Rht-B1a	9	77.11±1.38	20.17±3.14	31.18±0.99
Vida*4//Duclair/MALP 1930	Rht-A1-A53V	Rht-B1b	11	70.36±0.87	14.69±1.17	32.82±0.36
Vida*4//Duclair/MALP 1930	Rht-A1a	Rht-B1b	9	69.33±1.04	14.71±2.13	29.34±2.66
Vida*4//Duclair/MALP 1930	Rht-A1-A53V	Rht-B1a	6	83.5±1.65	15.57±1.48	32.33±0.63
Vida*4//Duclair/MALP 1930	Rht-A1a	Rht-B1a	13	82.62±0.84	17.16±1.16	33.09±0.3
Vida*4//Duclair/MALP 2141	Rht-A1-A53T	Rht-B1b	15	72.8±1.54	18.1±1.05	31.85±0.59
Vida*4//Duclair/MALP 2141	Rht-A1a	Rht-B1b	9	69.78±0.81	16.63±1.41	30.56±0.95
Vida*4//Duclair/MALP 2141	Rht-A1-A53T	Rht-B1a	10	82.1±1.51	16.96±1.12	33.2±0.41
Vida*4//Duclair/MALP 2141	Rht-A1a	Rht-B1a	14	82.14±1.19	17.62±1.93	31.95±1.21

[a] MALP is short for mutagenized Alpowa

[b] n represents the number of plants in each genotypic group.

[c] Values represent the mean ± the standard error

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively.

Table 3.3: Impact of Rht-A mutations on genotype class mean values in Duclair for agronomic traits in Bozeman 2022 field trials.

Cross[a]	Rht-A Genotype	Rht-B Genotype	n[b]	Plant Height[c] cm	Total Grain Yield[c] g/plant	Thousand Kernal Weight[c] mg/seed
Duclair*5/MALP 1158	Rht-A1-Q6	Rht-B1b	6	66.67±1.74	12.97±1.1	30.82±1.34
Duclair*5/MALP 1158	Rht-A1a	Rht-B1b	10	64.40±0.97	12.1±1.83	31.47±0.95
Duclair*5/MALP 1158	Rht-A1-Q6	Rht-B1a	12	73.42±2.14	12.38±1.68	33.73±0.81
Duclair*5/MALP 1158	Rht-A1a	Rht-B1a	6	76.00±1.77	12.84±4.1	34.45±0.67
Duclair*5/MALP 1305	Rht-A1-P94L	Rht-B1b	9	66.11±1.42	10.03±1.11	30.05±1.64
Duclair*5/MALP 1305	Rht-A1a	Rht-B1b	7	68.14±1.44	14.67±1.12	28.8±1.83
Duclair*5/MALP 1305	Rht-A1-P94L	Rht-B1a	17	78.12±0.53	11.15±0.9	33.74±2.38
Duclair*5/MALP 1305	Rht-A1a	Rht-B1a	13	77.54±0.94	11.69±1.29	31.08±0.69
Duclair*5/MALP 1461	Rht-A1-E63K	Rht-B1b	9	66.44±1.02	14.09±1.47***	32.37±0.72
Duclair*5/MALP 1461	Rht-A1a	Rht-B1b	13	66.46±0.87	15.82±1.55	32.64±0.82
Duclair*5/MALP 1461	Rht-A1-E63K	Rht-B1a	13	72.15±0.55**	14.89±1.12	31.14±0.94
Duclair*5/MALP 1461	Rht-A1a	Rht-B1a	12	75.33±0.89	13.34±1.46	30.61±0.76
Duclair*5/MALP 1487	Rht-A1-V55M	Rht-B1b	12	67.92±0.99	15.22±1.06	31.4±0.59
Duclair*5/MALP 1487	Rht-A1a	Rht-B1b	15	68.47±0.53	14.41±1.24	31±0.99
Duclair*5/MALP 1487	Rht-A1-V55M	Rht-B1a	15	78.33±1.04	14.79±0.75	32.44±0.75
Duclair*5/MALP 1487	Rht-A1a	Rht-B1a	6	77.33±0.61	12.88±1.04	32.33±0.93
Duclair*5/MALP 1536	Rht-A1-R3C	Rht-B1b	15	71.33±0.82	11.52±0.93	33.05±0.58
Duclair*5/MALP 1536	Rht-A1a	Rht-B1b	11	70.0±1.42	10.16±1.33	32.21±0.97
Duclair*5/MALP 1536	Rht-A1-R3C	Rht-B1a	18	79.94±0.99	13.7±1.26	33.04±0.52
Duclair*5/MALP 1536	Rht-A1a	Rht-B1a	21	78.67±1.05	12.7±0.82	32.97±0.53
Duclair*5/MALP 1930	Rht-A1-A53V	Rht-B1b	6	65.17±1.22	13.32±1.75	32.14±0.77
Duclair*5/MALP 1930	Rht-A1a	Rht-B1b	14	64.79±1.17	12.24±0.61	32.63±1.02
Duclair*5/MALP 1930	Rht-A1-A53V	Rht-B1a	8	73.75±0.9	13.81±1.77	30.94±0.56
Duclair*5/MALP 1930	Rht-A1a	Rht-B1a	15	72.47±0.98	11.24±0.72	31.91±0.66
Duclair*5/MALP 2141	Rht-A1-A53T	Rht-B1b	9	63.22±0.62	12.67±1.35	31.34±0.81*
Duclair*5/MALP 2141	Rht-A1a	Rht-B1b	11	64.82±1.12	12.08±0.91	28.14±0.98
Duclair*5/MALP 2141	Rht-A1-A53T	Rht-B1a	6	70.67±1.15	13.53±1.08	31.44±1.02
Duclair*5/MALP 2141	Rht-A1a	Rht-B1a	8	70.5±1.77	11.88±1.28	27.77±1.08

[a] MALP is short for mutagenized Alpowa

[b] n represents the number of plants in each genotypic group.

[c] Values represent the mean ± the standard error

*, **, and *** denote P values of <0.05, 0.01, and 0.001, respectively

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