N-terminal Rht-A1 mutations modify spring wheat (Triticum aestivum L.) plant height

Caleb O. Hale; Josey M. Ugrin; McKenna M. Volkman; Emma M. Tracy; John M. Martin; Andrew C. Hogg; Michael J. Giroux

Received: 31 July 2025 | Accepted: 4 March 2026

DOI: 10.1002/csc2.70279

Crop Science

ORIGINAL ARTICLE

Crop Breeding & Genetics

N-terminal *Rht-A1* mutations modify spring wheat (*Triticum aestivum* L.) plant height

Caleb O. Hale | **Josey M. Ugrin** | **McKenna M. Volkman** | **Emma M. Tracy** | **John M. Martin** | **Andrew C. Hogg** | **Michael J. Giroux**

Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, Montana, USA

Correspondence
Michael J. Giroux, Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT, USA.
Email: mgiroux@montana.edu

Assigned to Associate Editor Cankui Zhang.

Present addresses
Caleb O. Hale, Department of Crop and Soil Science, Oregon State University, Corvallis, OR, USA.

Josey M. Ugrin, CSS Farms LLC, Holbrook, ID, USA.

McKenna M. Volkman, Central Agricultural Research Station, Montana State University, Moccasin, MT, USA.

Emma M. Tracy, Department of Plant Biology, University of Vermont, Burlington, VT, USA.

Funding information
Montana Agricultural Experiment Station; Montana Wheat and Barley Committee; National Institute of Food and Agriculture

Abstract

The *Reduced Height-1* (*Rht-1*) gene in wheat (*Triticum aestivum* L.) has been well studied since the introgression of semidwarfing alleles *Rht-B1b* and *Rht-D1b* into many wheat varieties in the mid-20th century. *Rht-B1b* and *Rht-D1b* increase plant productivity but also reduce single grain weight and protein content. Other alleles of *Rht-1* as well as other height-affecting genes have been studied to provide breeders with alternative ways to modify plant height. To find *Rht-A1* alleles that could be used to fine tune plant height and other phenotypic traits that are affected by *Rht-1*, an ethyl methanesulfonate mutagenized Alpowa spring wheat population was screened for *Rht-A1* N-terminal mutations. Nine mutations were identified and characterized through yeast two-hybrid experiments to measure the impact each mutation had upon interaction with the gibberellin receptor GID1. Near isogenic lines were created by backcrossing the nine alleles into the cultivars Vida and Duclair, both with and without the semidwarfing allele *Rht-B1b*, and tested in preliminary spaced-plant trials. Five of these *Rht-A1* alleles (including a nonsense mutation allele, *Rht-A1*-Q6*) were selected for inclusion in full density yield trials. Only one mutant allele, *Rht-A1*-E63K, consistently decreased height across tested backgrounds. This included a height decrease of 6 cm and a yield increase of 12% in a "tall" background when no other height reducing alleles were present. The *Rht-A1*-E63K mutation occurs within the LExLE motif where *Rht-B1b* and *Rht-D1b* mutations also occur, emphasizing the importance of this locus to the function of *Rht-1*.

Plain Language Summary

The *Reduced Height-1* (*Rht-1*) gene in wheat controls plant height. While *Rht-B1b* and *Rht-D1b* versions boost yield, they also reduce seed size and protein. No dwarfing versions of the related *Rht-A1* gene had been found in hexaploid wheat. We used a

Abbreviations: EMS, ethyl methanesulfonate; GA3, gibberellic acid; NIL, near Isogenic line; PCR, polymerase chain reaction; Y2H, yeast two-hybrid.

mutagenized wheat population to find nine *Rht-A1* mutations and tested their effects. Five were selected for field trials. Only one, *Rht-A1*-E63K, consistently reduced plant height (by 6 cm) and increased yield (by 12%) in tall wheat without other dwarfing genes. This mutation occurs in a key region also affected in *Rht-B1b* and *Rht-D1b*.

1 | INTRODUCTION

Since the 1960s, the *Rht-B1b* and *Rht-D1b* alleles of the *Reduced Height-1* (*Rht-1*) gene have been integrated into all major wheat (*Triticum aestivum* L.) breeding programs. This was an extremely important component of the Green Revolution in the mid-20th century when the use of synthetic fertilizers became commonplace. These advancements allowed wheat crops to grow more vigorously and produce higher grain yields without lodging. Semidwarf wheat varieties carrying the *Rht-B1b* and *Rht-D1b* alleles were less likely to lodge and were more efficient with resource allocation (Hedden, 2003; Hoogendoorn et al., 1990). Lines carrying these alleles also on average have higher grain yields, more tillers, and produce grain with superior baking qualities (Jobson et al., 2018; Lanning et al., 2012). The *Rht-B1b* and *Rht-D1b* alleles originate from the Japanese variety Norin 10 and were popularized through the work of Norman Borlaug at CIMMYT. They are now known to be gain of function mutations that create stop codons in the *Rht-1* N-terminal region but experience some transcriptional reinitiation, ultimately creating low expression levels of a novel truncated protein that results in stem tissue-specific gibberellic acid (GA3) insensitivity (Gale & Marshall, 1976; Peng et al., 1999; Reynolds & Borlaug, 2006; Van De Velde et al., 2021). Despite their beneficial gain of function phenotypes, semidwarf lines carrying these alleles have some negative qualities, including reduced grain protein content, reduced single grain weight, increased susceptibility to Fusarium head blight (caused by *Fusarium graminearum* L.), reduced yields in extreme drought environments, and shorter coleoptile lengths resulting in poor emergence if seeds are planted deeply (Jobson et al., 2019; Mathews et al., 2006; Schillinger et al., 1998).

Research has been conducted on alternative *Rht* genes that also reduce height but do not encode DELLA proteins, including *Rht-8* and others (Casebow et al., 2016; Zhou et al., 2023). Additionally, a large body of research has also been based on the discovery and characterization of alternative *Rht-1* alleles that function differently from traditional semidwarfing alleles *Rht-B1b* and *Rht-D1b*. These studies have given us general insight into how *Rht* genes function and has also provided insight on how DELLA protein encoding *Rht-1* genes regulates plant growth and development. From a practical perspective, reducing plant height and improving grain yields while retaining drought tolerance, seedling emergence, and protein content are the main goals of these studies (Mathews et al., 2006; Schillinger et al., 1998). In order for plant breeders to fully understand and manipulate height regulation, continued study of the DELLA encoding *Rht-1* genes is essential.

Rht-1 is a master regulator of plant growth. It is expressed at similar levels across its three homoeologs, *Rht-A1, Rht-B1*, and *Rht-D1*, with one copy in each of the three hexaploid wheat genomes (Pearce et al., 2011; Ugrin et al., 2023). There are two main regions of *Rht-1*, each containing multiple conserved motifs (Figure 1). The GRAS domain, or C-terminal region, of *Rht-1* contains five conserved motifs and is responsible for interacting with and promoting the expression of downstream plant growth restricting genes and transcription factors (Wen et al., 2013; Zentella et al., 2007). The DELLA domain or N-terminal region of *Rht-1* contains three main conserved motifs, DELLA, LExLE, and TVHYNP, and is primarily responsible for binding with the gibberellin receptor GID1 (Murase et al., 2008; Pearce et al., 2011; Ueguchi-Tanaka et al., 2005). When GA3 is present, it forms a complex with GID1 and RHT-1, resulting in degradation of RHT-1 DELLA proteins themselves. Degradation of RHT-1 proteins effectively down-regulates growth restricting genes and other factors controlled by the C-terminal region of *Rht-1*, increasing stem elongation.

The phenotypic outcomes of mutations in *Rht-1* depend on their type and position. Nonsense mutations in *Rht-1* (except for those occurring in *Rht-B1b* and *Rht-D1b* alleles) generally decrease a plant's ability to regulate its height through the GA3 pathway, and plant height is increased. If all three *Rht-1* homoeologs are knocked out through nonsense mutations, a slender phenotype is observed with rapid growth and stem elongation (Ikeda et al., 2001; Ugrin et al., 2023). Missense mutations in the C-terminal of *Rht-1* can decrease the ability of *Rht-1* to promote growth restricting genes resulting in increased plant height. Missense mutations in the N-terminal region of *Rht-1* decrease the ability of GID1 to bind with RHT-1, decreasing the ability of GA3 to promote degradation of RHT-1 proteins and increasing the promotion of growth regulating genes, decreasing plant height (Chandler & Harding, 2013; Li et al., 2013). The *Rht-B1b* and *Rht-D1b* semidwarfing alleles have nonsense mutations that create stop codons in the LExLE motif in the N-terminal of *Rht-1*. However, partial translational reinitiation at a downstream AUG codon occurs constitutively in stem tissues, creating a

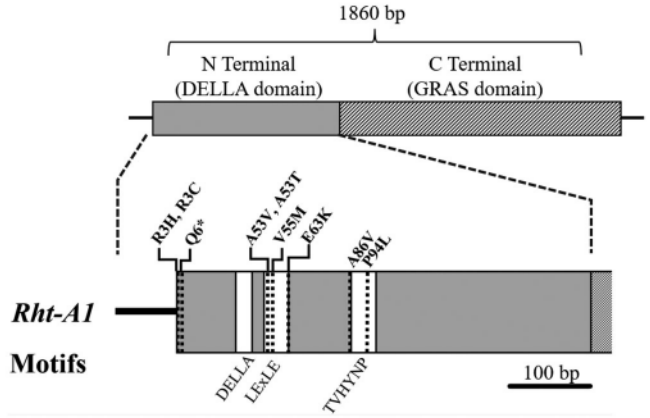

FIGURE 1 Visual representation of the N-terminal region of the *Rht-A1* gene, showing the location of discovered mutations discussed in this study. The E63K mutation located at the end of the LExLE domain had the greatest impact on plant height.

low level of N-terminally truncated DELLA proteins that are insensitive to GA3. These truncated proteins have the ability to promote growth restricting genes at a low level in stem tissues, and result in the semidwarf wheat phenotype (Van De Velde et al., 2021). A missense mutation in the *Rht-B1b* allele known as *Rht-B1b*-E529K partially decreases the ability of these truncated DELLA proteins to repress plant growth, and results in an intermediate semidwarf phenotype. Differences in phenotype resulting from a missense mutation that occurs after a nonsense mutation support the theory of transitional reinitiation in *Rht-B1b* (Brown et al., 2022; Mo et al., 2018). Another N-terminal mutation known as *Rht-B1c* or the Tom Thumb mutation includes an insertion directly before the last amino acid in the DELLA motif that inhibits all interaction between RHT-B1 and GID1, impeding the ability of GA3 to degrade RHT-B1. These RHT-B1c proteins continue to actively promote growth restricting genes indefinitely and are expressed at higher levels than *Rht-B1b* and *Rht-D1b*, resulting in an extreme dwarf phenotype (Casebow et al., 2016; Pearce et al., 2011). Lines containing the *Rht-B1c* mutation also show upregulation of GATA-type transcription factors compared to wild type, in agreement with similar work in *Arabidopsis thaliana*. This shows that DELLA proteins in wheat inhibit growth by interacting with growth promoting transcription factors in addition to promotion of growth restricting genes (Richter et al., 2010; Wen et al., 2013).

Natural and mutagenesis derived alternative alleles of *Rht-1* other than *Rht-B1b* and *Rht-D1b* have been characterized in *Rht-B1* and *Rht-D1* homoeologs, but fewer studies have identified and examined mutant alleles of *Rht-A1* (Brown et al., 2022; Flintham et al., 1997; Mo et al., 2018; Ugrin et al., 2023). Tan et al. (2013) identified an interesting nonsense mutant allele in the LExLE motif of *Rht-A1*; however, no studies were done to explore potential effects of this mutation. Li et al. (2013) used EcoTILLING methods to identify six different naturally occurring *Rht-A1* mutant alleles (denoted *Rht-A1b-g*) in Chinese wheat micro-core collections. Only four of these mutations resulted in non-synonymous codon mutations, and just one of them occurred in the N-terminal. None were predicted have deleterious phenotypic effects based on SIFT scores and nuclear localization assays. Most recently, Xie et al. (2024) characterized an ethyl methanesulfonate (EMS)-induced *Rht-A1* mutation that induces a plant height reduction of up to 49% compared to genotypes containing wildtype alleles of all three *Rht-1* homoeologues. This missense mutant allele, denoted as *Rht-A1h*, results in a glutamic acid to glycine mutation at the 44th codon (E44G) which occurs within the DELLA motif. Xie et al. (2024) also identified a large fragment inversion containing the *Rht-A1h* allele in mutant lines, which they theorized could be affecting phenotype as well. Research on alternative *Rht-A1* alleles provides insight into alternative height reducing alleles that function similarly to but are unlinked from traditional semidwarfing alleles *Rht-B1b* and *Rht-D1b*.

This study focuses on *Rht-A1* alleles with mutations in the N-terminal to find novel alleles that impart a reduced height phenotype. Tests were done in semidwarf cultivars containing the green revolution allele *Rht-B1b*, as well as tall lines containing the wildtype *Rht-B1a* allele. Since tested *Rht-A1* alleles are on the A genome and unlinked to *Rht-B1* and *Rht-D1*, these alternative reduced height alleles can be combined with *Rht-B1b* and *Rht-D1b* or any alternative semidwarfing *Rht* alleles to provide a greater range of height phenotypes, enabling plant breeders to fine tune plant height. These alleles offer new opportunities to reduce plant height without compromising single grain weight or protein content and enable the development of intermediate height cultivars that balance out some of the agronomic advantages and disadvantages of semidwarf varieties.

2 | MATERIALS AND METHODS

2.1 | Allele creation

All novel alleles in this study originate from a mutagenized population created in the spring wheat cultivar Alpowa (PI 566596) using EMS as described in Feiz et al. (2009). Alpowa contains the semidwarfing allele *Rht-D1b* on the D genome and a wildtype *Rht-B1a* allele on the B genome. Two thousand M_2 derived lines from this population were screened for novel *Rht-A1* N-terminal mutations. A bulk DNA sample from three M_2 derived M_3 plants was extracted from leaf tissue. *Rht-A1* was amplified using the nested polymerase chain reaction (PCR) protocol described in Li et al. (2013), and PCR products were sequenced by Sanger sequencing (GENEWIZ, Inc.). All induced mutations

TABLE 1 Summary of tested Rht-A1 mutations, including location, predicted effect on protein function, and summary of yeast two-hybrid results.

				Interaction with GID1	
Mutant allele	**Mutation type**	**PROVEAN score[a]**	**Predicted effect**	**With GA3**	**Without GA3**
Rht-A1-R3C	Missense	−4.9	Deleterious	No	Intermediate
Rht-A1-R3H	Missense	−2.77	Deleterious	Yes	Yes
Rht-A1-Q6*	Nonsense	NA	Deleterious	No	No
Rht-A1-A53T	Missense	−2.73	Deleterious	Yes	Yes
Rht-A1-A53V	Missense	−2.74	Deleterious	Yes	Yes
Rht-A1-V55M	Missense	−2.32	Neutral	No	Yes
Rht-A1-E63K	Missense	−3.28	Deleterious	No	Yes
Rht-A1-A86V	Missense	−2.31	Neutral	No	Yes
Rht-A1-P94L	Missense	−8.81	Deleterious	No	Yes

Note: Lines lacking restoration of growth in the presence of gibberellic acid (GA3) was not always predictive of effects on plant height.
[a]PROVEAN score of less than −2.5 indicates that the mutation is predicted to be deleterious.

discovered in the N-terminal region of *Rht-A1* were analyzed using PROVEAN to predict whether they would be deleterious (Choi & Chan, 2015). Missense mutations with a PROVEAN score of less than −2.5 were predicted to be deleterious. Seven different mutations with a PROVEAN score of less than −2.5, along with two mutations with a PROVEAN score of between −2.5 and −2.3, were chosen for further analysis. These alleles included *Rht-A1*-R3C, *Rht-A1*-R3H, *Rht-A1*-Q6*, *Rht-A1*-A53T, *Rht-A1*-A53V, *Rht-A1*-V55M, *Rht-A1*-E63K, *Rht-A1*-A86V, and *Rht-A1*-P94L (Table 1; Figure 1).

2.2 | Yeast two-hybrid screen

To test how these nine mutant alleles interact with GID1 in vitro, yeast two-hybrid (Y2H) assays were conducted. Binding ability was tested in the presence and absence of GA3. The bait in this assay was each of the nine mutant *Rht-1* alleles ligated into the pGADb plasmids. *Rht-A1a*, *Rht-B1a*, and *Rht-D1a* were used as positive controls, and *Rht-B1b*-E529K, previously described in Brown et al. (2022), along with an empty pGAD vector, was used as a negative control. The prey was GAL1 ligated into pGBKT7 plasmids. The bait and prey vectors were co-transformed into the *Saccharomyces cerevisiae* strain Y2HGold using the Yeastmaker Yeast Transformation System 2 (Clontech) and the Matchmaker Gold Y2H System (Takara Bio Inc.). Transformed yeast was grown on SD/-L-T selectable media to select for successfully co-transformed cells. Single colonies were selected and grown in SD/-L-T broth overnight. Each strain was brought up to a standard dilution of an OD600 of approximately 0.8. These dilutions, along with serial dilutions of 1:10, 1:100, 1:1,000, and 1:10,000, were plated onto SD/-L-T control plates, SD/-L-T-A-H plates, and SD/-L-T-A-H containing 100 μM concentration of GA3.

2.3 | NIL creation

Near isogenic lines (NIL) were created to test the effect of the nine mutant alleles in direct comparisons both with and without the semidwarfing *Rht-B1b* allele. M_3 Alpowa lines containing each of the nine mutant *Rht-A1* alleles along with the wildtype *Rht-B1a* allele were crossed to Duclair (PI 660981) (Lanning et al., 2011). F_1 progeny from these nine crosses were backcrossed to Duclair and also crossed to Vida (PI642366) (Lanning et al., 2006). Vida and Duclair are both high-yielding, Montana-adapted, hard red spring wheat varieties that contain the semidwarfing *Rht-B1b* allele. Lines were backcrossed with Duclair a total of five times and with Vida a total of four times. Plants were genotyped at each step by sanger sequencing using the protocol from Li et al. (2013). F_1 progeny were selected from each backcross that were heterozygous for their respective mutant *Rht-A1* allele and wildtype *Rht-B1a* allele and that were also heterozygous for the semidwarfing *Rht-B1b* allele native to Vida and Duclair and wildtype *Rht-B1a* allele from Alpowa. Duclair background lines containing the *Rht-A1*-A86V mutation and the *Rht-A1*-R3H mutation were lost during the backcrossing process. Two plants that were heterozygous for *Rht-A1* and *Rht-B1* were selected from each of the seven remaining genotype groups in Duclair and the nine genotype groups in Vida to make a total of 16 populations. About 200 BC_5F_2 (Duclair background) or BC_4F_2 (Vida background) seeds from each of these populations were hand planted in a spaced plant field experiment at the Post Agronomy Farm in Bozeman, MT, in 2022.

DNA samples were taken from each single plant in the 2022 field experiment and genotyped for *Rht-A1* and *Rht-B1*. Plants that were homozygous at both loci were selected to be phenotyped and harvested for advancement. This process created tall Duclair and tall Vida lines that included an introgressed wildtype *Rht-B1a* allele, along with standard semidwarf Duclair and semidwarf Vida lines that possessed the *Rht-B1b* allele. In this way, *Rht-A1* mutant alleles were tested within genetically isogenic tall and semidwarf backgrounds in two different varieties. Seeds were planted 15 cm apart in 3 m long rows with 30 cm spacing between each row. Handline sprinklers were used to apply 5 cm of irrigation water on June 21, June 26, and July 2. From May 1, 2022 to August 31, 2022, this location received 19.8 cm of precipitation (NCEI, 2025).

2.4 | Field experiments

In 2023 and 2024, field trials were planted at the Post Agronomy Farm near Bozeman, MT. Appropriate fertilizers and herbicides were applied to trials based on field conditions. Seeding rate for these experiments was approximately 308 seeds per m^2. Five of the nine EMS-induced *Rht-A1* mutation-carrying alleles were chosen for these trials based on the results of single plant measurements in 2022 and Y2H assays. Tested alleles were *Rht-A1*-Q6*, *Rht-A1*-E63K, *Rht-A1*-V55M, *Rht-A1*-R3C, and *Rht-A1*-A53T.

In 2023, this trial was arranged in a split plot design with a single replication because seed was limited. Each background and tested mutation combinations were the main plots and individual NIL as the subplots. NIL in the Duclair and Vida backgrounds were blocked as separate experiments. The trial included six randomly selected BC_5F_2 or BC_4F_2 derived isolines from each genotype group, except for the tall Vida *Rht-A1*-Q6* allele lines, which had a sample size of four. Two hundred sixty total plots were grown, including 120 Duclair background NIL, 117 Vida NIL, and parental checks. Plots were single 10 m rows spaced 30 cm apart. This trial was planted on May 4, 2023, and harvested on September 22, 2023. Plots were harvested with a single-row binder and threshed using a Vogel thresher. This experiment did not receive any supplemental irrigation. Between May 1, 2023 and August 1, 2023, this location received 22.0 cm of precipitation (NCEI, 2025).

In 2024, two NILs were randomly selected from each group representing every background, *Rht-B1* allele, and tested mutation (wildtype or mutant). NIL from Duclair and Vida backgrounds was blocked separately in these trials. Trials were arranged in a split-split plot randomized block design with two replications, with each tested mutation as the main plot, *Rht-B1* genotype as the subplot, and individual NIL as the sub-subplot. The same trial was grown in a rain-fed and an irrigated environment. With this design, shading effects on semidwarf lines from taller neighbors along with the effects of in-field variability were reduced. Individual plot size for 2024 experiments was 2 m^2, consisting of two 10 m long rows spaced 30 cm apart. The rainfed trial was planted on 16 April 2024, and the irrigated experiment was planted April 24, 2024. Plots were harvested using a small plot combine harvester. The irrigated trial utilized handline irrigation equipment, applying approximately 5 cm of supplemental water around flowering. Between April 1 and August 1, 2024, this location received 20.3 cm of precipitation (NCEI, 2025).

2.5 | Phenotyping

In the 2022 single plant experiment, plant height was measured from the soil surface to the tip of the tallest head (excluding awns). Yield was measured in grams as the total weight of the grain produced from each individual plant. In 2023 and 2024, two representative areas were selected in each plot to account for potential spatial variability. Areas were visually chosen to represent typical plant growth and to avoid plot edges or visibly uneven patches. Within each area, plant height was measured from the soil surface to the tip of the tallest head (excluding awns). Plot yield was calculated using the total grain weight from each plot and the plot area to determine equivalent grain yield in kilograms per hectare. Each year, single grain weight was calculated by manually counting 200 seeds, weighing them, and calculating an average. Grain protein was measured by near-infrared transmittance using a Foss Infratec 1241 Grain Analyzer (Foss North America).

2.6 | Statistical analysis

In the single plant trials, comparisons between mutant and wild-type means in each cross were made using two-tailed, independent sample t-tests. Data from the two NILs that were grown in both years of full density field trials was compiled, and mean values of the NIL representing each genotype group were calculated and used for further analysis. Data from each of the 10 different populations representing each height/variety background grown in the full density trials was analyzed separately. Analysis of variance was computed using a mixed linear model with *Rht-A1* genotype, *Rht-B1* genotype, environment, and their interactions as fixed effects. The interaction between replicate and environment was considered as a random effect. Assumptions of normality and homogeneity of variance were verified prior to model fitting. The 2023 rainfed, 2024 irrigated, and 2024 rainfed experiments were considered three separate environments. Analysis was performed using the lme4 package (Bates

et al., 2015) in R (R Foundation for Statistical Computing, Version 4.0.5).

3 | RESULTS AND DISCUSSION

3.1 | Y2H screen

Strains transformed with *Rht-A1*a, *Rht-B1a*, and *Rht-D1a* positive controls showed vigorous growth, indicating strong interaction with GID1 in both test plates, with and without GA3. Negative controls *Rht-B1b*-E529K and GID1/empty vector did not show any interaction. The single nonsense *Rht-A1* allele and eight different missense *Rht-A1* alleles were observed to interact with GID1 in three different ways. *Rht-A1*-Q6* interacted with GID1 similarly to the negative controls, with no growth observed in the presence or absence of GA3. Both *Rht-A1*-A53T and *Rht-A1*-R3C grew less than any other tested strains on the control plate, but *Rht-A1*-A53T showed interaction similar to positive controls with and without GA3, while *Rht-A1*-R3C appeared to have little to no interaction without GA3 but partially restored interaction in the presence of GA3. *Rht-A1*-A86V, *Rht-A1*-P94L, *Rht-A1*-V55M, and *Rht-A1*-E63K showed no interaction with GID1 without GA3, but interaction with GID1 was restored in the presence of GA3. *Rht-A1*-A53V and *Rht-A1*-R3H performed similarly to positive controls and interacted with GID1 in both the presence and absence of GA3 (see Figure 2).

3.2 | Single plant study

The *Rht-A1*-V55M allele decreased height significantly in the semidwarf Vida background by 4.4% compared to the wild type ($p = 0.01$) (Table S1), and the *Rht-A1*-E63K allele decreased height in the tall Duclair background by 4.2% compared to the wild type ($p = 0.01$) (Table S2). *Rht-A1*-A53T showed increased single grain weight in all four comparisons, with a significant increase of 10.2% in the semidwarf Duclair background ($p = 0.02$). No other significant differences in height were detected in the single plant study. These results contributed to these lines being chosen for inclusion in the full density trials. See supporting information tables for mean values of all data collected in the preliminary single spaced plant trials.

3.3 | Standard planting density field study

Each tested *Rht-A1* mutation included in the full density trials contributed to a small decrease in height in both semidwarf and tall backgrounds (Table 2). This difference was most pronounced in the *Rht-A1*-E63K allele Vida background comparisons, in which this allele decreased height by about 6 cm in both tall and semidwarf backgrounds ($p < 0.01$). On average, *Rht-A1* mutant alleles decreased grain yield in semidwarf backgrounds but increased grain yield in tall backgrounds. The only statistically significant grain yield difference was in a tall Vida background comparison in which the *Rht-A1*-E63K allele increased grain yield by 12% ($p = 0.02$). Protein content was not affected by *Rht-A1* mutations with one exception, where there was a significant protein content decrease in which the *Rht-A1*-R3C allele decreased protein content by 2.6% ($p = 0.04$) in a tall Duclair background. As in the single plant study, lines with the *Rht-A1*-A53T mutation all trended toward an increase in single grain weight, with the tall Duclair background lines showing a statistically significant increase of 2.6% ($p = 0.01$). Most other *Rht-A1* mutations contributed toward a decrease in single grain weight, with this decrease being statistically significant in the tall Vida *Rht-A1*-R3C comparison ($p = 0.05$), and the tall Duclair *Rht-A1*-V55M comparison ($p = 0.02$). All semidwarf background lines were between 15 and 19 cm shorter on average than tall background lines. In general, semidwarf lines also had higher grain yield but lower protein content and single grain weight (Table 2).

4 | DISCUSSION

Although the *Rht-A1*-Q6* allele performed similarly to the control *Rht-B1b*-E529K allele in Y2H assays, lines with this allele did not show an impact on plant height or seed metrics like the *Rht-B1b* or *Rht-D1b* alleles. These results suggest that this nonsense mutant allele does not have similar function to the green revolution semi-dwarfing alleles *Rht-B1b* and *Rht-D1b*, which also contain stop codons. *Rht-B1b* and *Rht-D1b* experience tissue specific translational reinitiation after the stop mutation to create an N-terminally truncated protein, resulting in a semidwarf phenotype. However, the *Rht-A1*-Q6* allele appears not to experience this specific form of translational reinitiation since it does not confer a similar semi-dwarfing effect. In contrast, other tested missense alleles exhibited reductions in plant height, notably the *Rht-A1*-E63K mutation allele. This partial effect, coupled with restored Y2H interaction in the presence of GA3, suggests that this variant may retain some GA-dependent binding activity in planta, potentially altering GA sensitivity without fully disrupting DELLA degradation. Results of this study show a weak correlation between tested Y2H interactions and field phenotypes, indicating that Y2H assays alone are not a sufficient predictor of plant height. These findings emphasize that multiple regulatory mechanisms contribute to the phenotypic expression of *Rht-1* variants, not simply the binding ability of RHT-1 with GID1.

TABLE 2 Full density field trial data averaged across 2023 and 2024 environments.

Tested *Rht-A1* allele	Variety	*Rht-B1*	*Rht-A1*	Height (cm)	Grain yield (kg ha^{-1})	Grain protein (g kg^{-1})	Single grain weight (mg)
Rht-A1-R3C	Duclair	Semidwarf[a]	*Rht-A1*-R3C	90.3 ± 1.2	5719 ± 178	146 ± 1.2	33.1 ± 0.5
			Rht-A1a	90.3 ± 1.2	5838 ± 178	146 ± 1.2	33.5 ± 0.5
		Tall[b]	*Rht-A1*-R3C	105.2* ± 1.2	5757 ± 178	149* ± 1.2	33.9 ± 0.5
			Rht-A1a	107.7 ± 1.2	5453 ± 178	153 ± 1.2	33.2 ± 0.5
	Vida	Semidwarf	*Rht-A1*-R3C	87 ± 0.67	5759 ± 158	149 ± 2.7	32.5 ± 0.4
			Rht-A1a	87.8 ± 0.7	5845 ± 158	147 ± 2.7	31.5 ± 0.4
		Tall	*Rht-A1*-R3C	105.5* ± 0.7	5542 ± 158	154 ± 2.7	32.3* ± 0.4
			Rht-A1a	107.7 ± 0.7	5372 ± 158	157 ± 2.7	33.5 ± 0.4
Rht-A1-Q6*	Duclair	Semidwarf	*Rht-A1*-Q6*	84.5 ± 1.4	5994 ± 330	148 ± 1.9	31.7 ± 0.7
			Rht-A1a	87 ± 1.4	6213 ± 330	146 ± 1.9	32.4 ± 0.7
		Tall	*Rht-A1*-Q6*	102.1 ± 1.4	5799 ± 330	150 ± 1.9	34.4 ± 0.7
			Rht-A1a	101.3 ± 1.4	5856 ± 330	154 ± 1.9	32.8 ± 0.7
	Vida	Semidwarf	*Rht-A1*-Q6*	87.5* ± 0.8	5790 ± 354	145 ± 1.5	32 ± 0.9
			Rht-A1a	90.2 ± 0.8	5911 ± 354	141 ± 1.5	32 ± 0.9
		Tall	*Rht-A1*-Q6*	106.2 ± 0.8	5524 ± 354	147 ± 1.5	32.8 ± 0.9
			Rht-A1a	106.8 ± 0.8	5294 ± 354	148 ± 1.5	34.3 ± 0.9
Rht-A1-A53T	Duclair	Semidwarf	*Rht-A1*-A53T	85.4 ± 1.1	6167 ± 465	147 ± 3.3	30.9 ± 0.9
			Rht-A1a	85 ± 1.1	6472 ± 465	145 ± 3.3	30.6 ± 0.9
		Tall	*Rht-A1*-A53T	100.4 ± 1.1	5924 ± 465	150 ± 3.3	32.0* ± 0.9
			Rht-A1a	101.2 ± 1.1	5979 ± 465	151 ± 3.3	31.2 ± 0.9
	Vida	Semidwarf	*Rht-A1*-A53T	87.9 ± 1.0	6060 ± 349	140 ± 2.1	32.5 ± 0.4
			Rht-A1a	90.2 ± 1.0	6011 ± 349	141 ± 2.1	32.2 ± 0.4
		Tall	*Rht-A1*-A53T	106.8 ± 1.0	5440 ± 349	143 ± 2.1	34.6 ± 0.4
			Rht-A1a	108.2 ± 1.0	5069 ± 349	146 ± 2.1	33.9 ± 0.4
Rht-A1-V55M	Duclair	Semidwarf	*Rht-A1*-V55M	85.2 ± 1.5	5587 ± 346	148 ± 3.0	31 ± 0.6
			Rht-A1a	86.6 ± 1.5	5559 ± 351	149 ± 3.0	31.9 ± 0.6
		Tall	*Rht-A1*-V55M	101.8 ± 1.5	5463 ± 346	155 ± 3.0	31.7* ± 0.6
			Rht-A1a	102.2 ± 1.5	5666 ± 346	151 ± 3.0	33.2 ± 0.6
	Vida	Semidwarf	*Rht-A1*-V55M	86.8* ± 1.3	5832 ± 342	139 ± 2.5	32.8 ± 0.7
			Rht-A1a	89.6 ± 1.3	6198 ± 322	140 ± 2.5	33.8 ± 0.7
		Tall	*Rht-A1*-V55M	107.3 ± 1.3	5469 ± 322	148 ± 2.5	34 ± 0.7
			Rht-A1a	108.3 ± 1.3	5983 ± 322	146 ± 2.5	34.5 ± 0.7
Rht-A1-E63K	Duclair	Semidwarf	*Rht-A1*-E63K	83.5* ± 1.1	6340 ± 281	143 ± 1.2	31.9 ± 0.4
			Rht-A1a	87.4 ± 1.1	6489 ± 281	145 ± 1.2	32.8 ± 0.4
		Tall	*Rht-A1*-E63K	94.8 ± 1.1	6287 ± 281	148 ± 1.2	32 ± 0.4
			Rht-A1a	96.7 ± 1.1	5895 ± 281	151 ± 1.2	31 ± 0.4
	Vida	Semidwarf	*Rht-A1*-E63K	84.9** ± 1.1	6645 ± 208	141 ± 1.8	27 ± 1.5
			Rht-A1a	91 ± 1.1	6637 ± 208	141 ± 1.6	30.5 ± 1.5
		Tall	*Rht-A1*-E63K	103.1** ± 1.1	6046* ± 208	144 ± 1.6	31.9 ± 1.5
			Rht-A1a	108.9 ± 1.1	5398 ± 208	147 ± 1.6	32.7 ± 1.5

Note: Mean values and standard errors are shown. The Rht-A1-E63K mutation had the largest effect on plant height and grain yield.

[a]Semidwarf lines contain the *Rht-B1b* allele.

[b]Tall lines contain the *Rht-B1a* allele.

* and **denote significant difference at 0.05 and 0.01 probability levels.

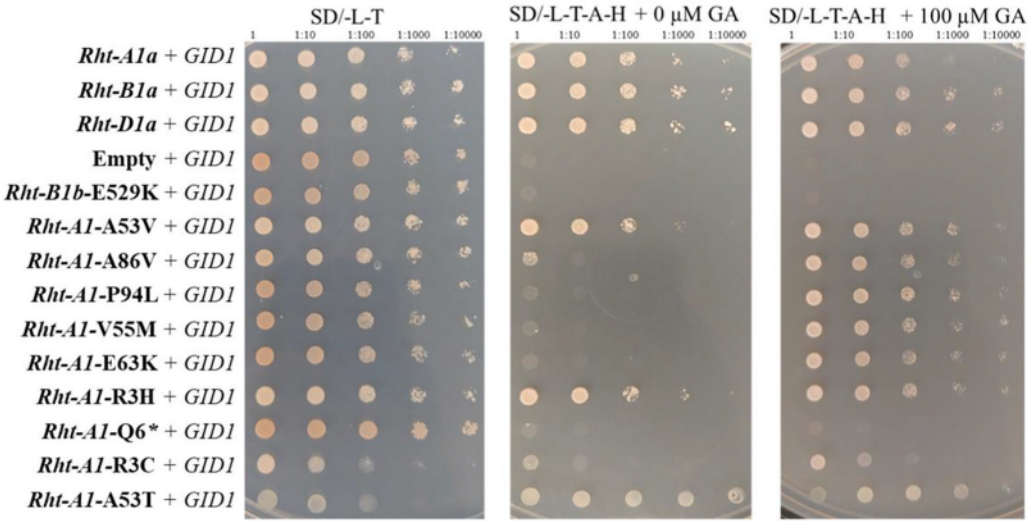

FIGURE 2 Yeast two-hybrid assay analyzing *Rht-1* mutant alleles found in hexaploid wheat indicating interaction between RHT-1 and GID1 in the presence or absence of 100 µM gibberellic acid (GA3). Lines lacking restoration of growth in the presence of GA3 was not always predictive of effects on plant height. Serial dilutions on SD/-L-T (control) media, SD/-L-T-A-H media, and SD/-L-T-A-H media + 100 µM GA3. *Rht-A1a*, *Rht-B1a, and Rht-D1a* are positive controls, *Rht-B1b-E529K* is a negative control, and GID1 is a negative control with an empty pGADT7 vector. Plates are representative of three independent replications. *Rht-A1-A86V, Rht-A1-P94L*, *Rht-A1-V55M*, and *Rht-A1-E63K* did not grow on the no-GA3 plate. The addition of GA3 restored the interaction of *Rht-A1-A53V*, *Rht-A1-A86V*, *Rht-A1-P94L*, *Rht-A1-V55M*, *Rht-A1-E63K*, *Rht-A1-R3H*, and *Rht-A1-A53T* to the same level as the wild-type positive controls. *Rht-A1-Q6** mimicked the negative controls, with no growth on either plate. Both *Rht-A1-A53T* and *Rht-A1-R3C* had reduced growth on the control plate, but in comparing the interaction plates still showed enough growth to interpret.

The *Rht-A1*-E63K missense mutation allele conferred the largest height and yield differences in both the single plant and the full density yield trials. Out of all tested mutant alleles, this mutation is closest to the corresponding homologous location of the nonsense mutations in the *Rht-B1b* (Q64*) and *Rht-D1b* (E61*) alleles. Each of these three mutations occur directly within the eponymous portion of the LExLE motif, which is located between the 52nd and 66th amino acid in *Rht-A1*, the 55th and 69th amino acid in *Rht-B1*, and the 53rd and 67th amino acid in *Rht-D1*. *Rht-A1*-E63K changes the second glutamic acid codon to a lysine codon, *Rht-B1b* changes the glutamine codon (between the leucine/glutamine codon pairs) to a stop codon, and *Rht-D1b* changes the first glutamic acid codon to a stop codon. Three of the other alleles in this study have mutations that occur within this motif but before the eponymous portion, *Rht-A1*-A53T, *Rht-A1*-A53V, and *Rht-A1*-V55M. Although these mutations trended toward reducing height, they did not impact height, yield, or seed metrics to the same degree as *Rht-A1*-E63K. This adds to the evidence that the stop mutations found in *Rht-B1b* and *Rht-D1b* alleles occur in a novel location, resulting in the rare transcription reinitiation described in Van De Velde et al. (2021) and the gain of function traits that we see in semidwarf wheat cultivars. Additionally, the results of this study are supported by Murase et al. (2008), which describes the importance of this specific locus in DELLA proteins in the orthologous *GA3 Insensitive (GAI)* gene in *Arabidopsis thaliana*. Their model of the GAI DELLA protein, which contains the DELLA, TVHYNP, and LExLE motifs, puts special emphasis on the glutamic acid codons of the LExLE motif, showing that these negatively charged residues directly stabilize binding with the GA3 GID1 complex through salt bridges formed with positive amino acid residues in GID1. Results from a pull-down binding assay that tested binding ability of various GAI mutants with GA3 bound GID1A showed that the *GAI*-E54R mutation (directly homologous in location with the *Rht-A1*-E63K mutation in this study) reduced binding more so than any other tested single point mutation (Murase et al., 2008).

Xie et al. (2024) identified the *Rht-A1h* mutant allele (E44G), which occurs within the DELLA motif two amino acids after the eponymous portion of this motif, which is located between the 38th and 42nd amino acid in *Rht-A1*. This study did not examine any mutations occurring in the DELLA motif of *Rht-A1*. Altogether, the results of this study together with the results of Xie et al. (2024) reinforce research showing that the DELLA and LExLE motifs within the N-terminal region or DELLA domain of *Rht-1* are especially important in the function of *Rht-1* in wheat.

The *Rht-A1*-E63K allele shows agronomic value when compared to tall background genotypes and can realistically be integrated into breeding programs in which mid height varieties may be beneficial. Wheat varieties with taller plant height improves increased weed competitive ability, which

is useful in organic systems and other systems wanting to implement integrated weed management practices (Andrew et al., 2015; Lazzaro et al., 2019; Murphy et al., 2008). Lines that contain *Rht-A1*-E63K allele grown in these types of systems would potentially show improvements in yield over full height lines but would likely retain the weed competitive ability of a taller line compared to a semidwarf variety. In conventional systems, mid height lines containing only the *Rht-A1*-E63K allele may show improvements in aforementioned negative qualities conferred by semidwarfing alleles *Rht-B1b and Rht-D1b*. Although no significant differences in protein content were detected in this study, increases in emergence due to longer coleoptile length, increased yields in extreme drought environments, and increased Fusarium head blight resistance may be conferred by *Rht-A1*-E63K relative to semidwarf genotypes (Jobson et al., 2019; Mathews et al., 2006; Schillinger et al., 1998). Further research is needed to determine whether these benefits are realized.

5 | CONCLUSION

The *Rht-A1*-E63K mutation decreased plant height in both tall and short backgrounds, although this difference was not statistically significant in the tall Duclair background. This mutation also significantly increased grain yield when tested in the tall Vida background without significantly decreasing grain protein content. Grain yield was also increased in the tall Duclair background, although this increase was not statistically significant. Other tested mutations, including the *Rht-A1*-Q6* nonsense mutation did not consistently impact plant height. This indicates that the region of LExLE motif where the E63K mutation and *Rht-D1b* and *Rht-B1b* allele mutations occur is especially integral to the function of *Rht-1* in determining plant height. Overall, this data supports the introgression of the *Rht-A1*-E63K allele into wheat breeding programs for the purpose of fine-tuning plant height for environments or situations that may warrant an intermediate height phenotype. This phenotype would be shorter than wild type (tall) lines but not as short as semidwarf lines carrying the traditional *Rht-B1b* or *Rht-D1b* alleles and would also not confer the significant seed quality drawbacks that these alleles bring.

AUTHOR CONTRIBUTIONS

Caleb O. Hale: Formal analysis; investigation; methodology; project administration; writing—original draft; writing—review and editing. **Josey M. Ugrin**: Conceptualization; formal analysis; methodology; supervision; writing—review and editing. **McKenna M. Volkman**: Formal analysis; investigation; methodology; writing—review and editing. **Emma M. Tracy**: Formal analysis; methodology; writing—review and editing. **John M. Martin**: Conceptualization; formal analysis; project administration; writing—review and editing. **Andrew C. Hogg**: Formal analysis; methodology; supervision; writing—review and editing. **Michael J. Giroux**: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; writing—original draft; writing—review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Caleb O. Hale https://orcid.org/0009-0000-3043-6284
John M. Martin https://orcid.org/0000-0001-8558-6482
Michael J. Giroux https://orcid.org/0000-0001-7343-6719

REFERENCES

Andrew, I. K. S., Storkey, J., & Sparkes, D. L. (2015). A review of the potential for competitive cereal cultivars as a tool in integrated weed management. *Weed Research*, *55*(3), 239–248. https://doi.org/10.1111/wre.12137

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Brown, M. M., Martin, J. M., Jobson, E. M., Hogg, A. C., Carr, P. M., & Giroux, M. J. (2022). Evaluating the impact of *Rht* hypomorphic mutations in durum wheat. *Crop Science*, *62*(1), 247–258. https://doi.org/10.1002/csc2.20672

Casebow, R., Hadley, C., Uppal, R., Addisu, M., Loddo, S., Kowalski, A., Griffiths, S., & Gooding, M. (2016). Reduced height (*Rht*) alleles affect wheat grain quality. *PLoS One*, *11*(5), e0156056. https://doi.org/10.1371/journal.pone.0156056

Chandler, P. M., & Harding, C. A. (2013). 'Overgrowth' mutants in barley and wheat: New alleles and phenotypes of the 'Green Revolution' *Della* gene. *Journal of Experimental Botany*, *64*(6), 1603–1613. https://doi.org/10.1093/jxb/ert022

Choi, Y., & Chan, A. P. (2015). PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*, *31*(16), 2745–2747. https://doi.org/10.1093/bioinformatics/btv195

Feiz, L., Beecher, B. S., Martin, J. M., & Giroux, M. J. (2009). *In planta* mutagenesis determines the functional regions of the wheat puroindoline proteins. *Genetics*, *183*(3), 853–860. https://doi.org/10.1534/genetics.109.106013

Flintham, J. E., Börner, A., Worland, A. J., & Gale, M. D. (1997). Optimizing wheat grain yield: Effects of *Rht* (gibberellin-insensitive) dwarfing genes. *Journal of Agricultural Science*, *128*, 11–25. https://doi.org/10.1017/S0021859696003942

Gale, M. D., & Marshall, G. A. (1976). Chromosomal location of *Gai*-1 and *Rht-1*, genes for gibberellin insensitivity and semi-dwarfism, in a derivative of Norin-10 wheat. *Heredity*, *37*(Oct), 283–289. https://doi.org/10.1038/hdy.1976.88

Hedden, P. (2003). The genes of the green revolution. *Trends in Genetics*, *19*(1), 5–9. https://doi.org/10.1016/s0168-9525(02)00009-4

Hoogendoorn, J., Rickson, J., & Gale, M. (1990). Differences in leaf and stem anatomy related to plant height of tall and dwarf wheat (*Triticum aestivum* L.). *Journal of Plant Physiology*, *136*(1), 72–77. https://doi.org/10.1016/S0176-1617(11)81618-4

Ikeda, A., Ueguchi-Tanaka, M., Sonoda, Y., Kitano, H., Koshioka, M., Futsuhara, Y., Matsuoka, M., & Yamaguchi, J. (2001). *slender* rice, a constitutive gibberellin response mutant, is caused by a null mutation of the *SLR1* gene, an ortholog of the height-regulating gene *GAI/RGA/RHT/D8*. *Plant Cell*, *13*(5), 999–1010. https://doi.org/10.1105/tpc.13.5.999

Jobson, E. M., Johnston, R. E., Oiestad, A. J., Martin, J. M., & Giroux, M. J. (2019). The impact of the wheat *Rht-B1b* semi-dwarfing allele on photosynthesis and seed development under field conditions. *Frontiers in Plant Science*, *10*, 51. https://doi.org/10.3389/fpls.2019.00051

Jobson, E. M., Martin, J. M., Schneider, T. M., & Giroux, M. J. (2018). The impact of the *Rht-B1b, Rht-D1b*, and *Rht-8* wheat semi-dwarfing genes on flour milling, baking, and micronutrients. *Cereal Chemistry*, *95*(6), 770–778. https://doi.org/10.1002/cche.10091

Lanning, S. P., Carlson, G. R., Lamb, P. F., Nash, D., Wichman, D. M., Kephart, K. D., Stougaard, R. N., Miller, J., Kushnak, G. D., Eckhoff, J. L., Grey, W. E., Blake, N. K., & Talbert, L. E. (2011). Registration of 'Duclair' hard red spring wheat. *Journal of Plant Registrations*, *5*(3), 349–352. https://doi.org/10.3198/jpr2011.02.0064crc

Lanning, S. P., Carlson, G. R., Nash, D., Wichman, D. M., Kephart, K. D., Stougaard, R. N., Kushnak, G. D., Eckhoff, J. L., Grey, W. E., Dyer, A., & Talbert, L. E. (2006). Registration of 'Vida' wheat. *Crop Science*, *46*(5), 2315–2316. https://doi.org/10.2135/cropsci2006.03.0167

Lanning, S. P., Martin, J. M., Stougaard, R. N., Guillen-Portal, F. R., Blake, N. K., Sherman, J. D., Robbins, A. M., Kephart, K. D., Lamb, P., Carlson, G. R., Pumphrey, M., & Talbert, L. E. (2012). Evaluation of near-isogenic lines for three height-reducing genes in hard red spring wheat. *Crop Science*, *52*(3), 1145–1152. https://doi.org/10.2135/cropsci2011.11.0625

Lazzaro, M., Bàrberi, P., Dell'Acqua, M., Pè, M. E., Limonta, M., Barabaschi, D., Cattivelli, L., Laino, P., & Vaccino, P. (2019). Unraveling diversity in wheat competitive ability traits can improve integrated weed management. *Agronomy for Sustainable Development*, *39*(1), Article 6. https://doi.org/10.1007/s13593-018-0551-1

Li, A., Yang, W., Lou, X., Liu, D., Sun, J., Guo, X., Wang, J., Li, Y., Zhan, K., Ling, H.-Q., & Zhang, A. (2013). Novel natural allelic variations at the *Rht-1* loci in wheat. *Journal of Integrative Plant Biology*, *55*(11), 1026–1037. https://doi.org/10.1111/jipb.12103

Mathews, K. L., Chapman, S. C., Trethowan, R., Singh, R. P., Crossa, J., Pfeiffer, W., Van Ginkel, M., & Delacy, I. (2006). Global adaptation of spring bread and durum wheat lines near-isogenic for major reduced height genes. *Crop Science*, *46*(2), 603–613. https://doi.org/10.2135/cropsci2005.05-0056

Mo, Y., Pearce, S., & Dubcovsky, J. (2018). Phenotypic and transcriptomic characterization of a wheat tall mutant carrying an induced mutation in the C-terminal PFYRE motif of RHT-B1b. *Bmc Plant Biology*, *18*, Article 25. https://doi.org/10.1186/s12870-018-1465-4

Murase, K., Hirano, Y., Sun, T. P., & Hakoshima, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature*, *456*(7221), 459–U415. https://doi.org/10.1038/nature07519

Murphy, K. M., Dawson, J. C., & Jones, S. S. (2008). Relationship among phenotypic growth traits, yield and weed suppression in spring wheat landraces and modern cultivars. *Field Crops Research*, *105*(1), 107–115. https://doi.org/10.1016/j.fcr.2007.08.004

NCEI. (2025). *Daily summaries station details*. National Climatic Data Center (NCDC). https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00241047/detail

Pearce, S., Saville, R., Vaughan, S. P., Chandler, P. M., Wilhelm, E. P., Sparks, C. A., Al-Kaff, N., Korolev, A., Boulton, M. I., Phillips, A. L., Hedden, P., Nicholson, P., & Thomas, S. G. (2011). Molecular characterization of *Rht-1* dwarfing genes in hexaploid wheat. *Plant Physiology*, *157*(4), 1820–1831. https://doi.org/10.1104/pp.111.183657

Peng, J., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., Pelica, F., Sudhakar, D., Christou, P., Snape, J. W., Gale, M. D., & Harberd, N. P. (1999). 'Green revolution' genes encode mutant gibberellin response modulators. *Nature*, *400*(6741), 256–261. https://doi.org/10.1038/22307

Reynolds, M. P., & Borlaug, N. E. (2006). Impacts of breeding on international collaborative wheat improvement. *Journal of Agricultural Science*, *144*, 3–17. https://doi.org/10.1017/S0021859606005867

Richter, R., Behringer, C., Müller, I. K., & Schwechheimer, C. (2010). The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS. *Genes & Development*, *24*(18), 2093–2104. https://doi.org/10.1101/gad.594910

Schillinger, W. F., Donaldson, E., Allan, R. E., & Jones, S. S. (1998). Winter wheat seedling emergence from deep sowing depths. *Agronomy Journal*, *90*(5), 582–586. https://doi.org/10.2134/agronj1998.00021962009000050002x

Tan, M.-K., Koval, J., & Ghalayini, A. (2013). Novel genetic variants of GA-insensitive *Rht-1* genes in hexaploid wheat and their potential agronomic value. *PLoS One*, *8*(7), e69690. https://doi.org/10.1371/journal.pone.0069690

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., Chow, T.-Y., Hsing, Y.-I. C., Kitano, H., Yamaguchi, I., & Matsuoka, M. (2005). *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. *Nature*, *437*(7059), 693–698. https://doi.org/10.1038/nature04028

Ugrin, J. M., Hogg, A. C., Tracy, E. M., Tillett, B. J., Cook, J. P., Martin, J. M., & Giroux, M. J. (2023). Dosage response to *reduced height-1* (*Rht-1*) loss-of-function mutations and characterization of slender phenotype in hexaploid wheat. *Crop Science*, *63*(6), 3457–3469. https://doi.org/10.1002/csc2.21116

Van De Velde, K., Thomas, S. G., Heyse, F., Kaspar, R., Van Der Straeten, D., & Rohde, A. (2021). N-terminal truncated RHT-1 proteins generated by translational reinitiation cause semi-dwarfing of wheat Green Revolution alleles. *Molecular Plant*, *14*(4), 679–687. https://doi.org/10.1016/j.molp.2021.01.002

Wen, W., Deng, Q., Jia, H., Wei, L., Wei, J., Wan, H., Yang, L., Cao, W., & Ma, Z. (2013). Sequence variations of the partially dominant DELLA gene *Rht-B1c* in wheat and their functional impacts. *Journal of Experimental Botany*, *64*(11), 3299–3312. https://doi.org/10.1093/jxb/ert183

Xie, X., Zhang, Y., Xu, L., Xiong, H., Xie, Y., Zhao, L., Gu, J., Li, H., Zhang, J., Ding, Y., Zhao, S., Guo, H., & Liu, L. (2024). Mapping of dwarfing gene and identification of mutant allele on plant height in wheat. *Molecular Breeding*, *44*(11), 79. https://doi.org/10.1007/s11032-024-01515-3

Zentella, R., Zhang, Z.-L., Park, M., Thomas, S. G., Endo, A., Murase, K., Fleet, C. M., Jikumaru, Y., Nambara, E., Kamiya, Y., & Sun, T.-P. (2007). Global analysis of DELLA direct targets in early gibberellin

signaling in. *Plant Cell*, *19*(10), 3037–3057. https://doi.org/10.1105/tpc.107.054999

Zhou, C., Xiong, H., Fu, M., Guo, H., Zhao, L., Xie, Y., Gu, J., Zhao, S., Ding, Y., Li, Y., Li, X., & Liu, L. (2023). Genetic mapping and identification of *Rht8-B1* that regulates plant height in wheat. *Bmc Plant Biology*, *23*(1), Article 333. https://doi.org/10.1186/s12870-023-04343-3

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hale, C. O, Ugrin, J. M, Volkman, M. M, Tracy, E. M, Martin, J. M, Hogg, A. C, & Giroux, M. J (2026). N-terminal *Rht-A1* mutations modify spring wheat (*Triticum aestivum* L.) plant height. *Crop Science*, *66*, e70279. https://doi.org/10.1002/csc2.70279