Rht-1 Semi-Dwarfing Alleles Increase the Abundance of High Molecular Weight Glutenin Subunits

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Rht-1 Semi-Dwarfing Alleles Increase the Abundance of High Molecular Weight Glutenin Subunits

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ABSTRACT

Background and Objectives: Grain protein and starch abundance and composition are quantitative traits that play key roles in wheat quality. The semi-dwarfing alleles of the *Reduced*

height (*Rht-1*) gene increase tillers and yield but also reduce seed size and protein content. Despite their negative impact on grain protein content the semi-dwarfing alleles increase dough mixing time and tolerance. This study used near isogenic lines that were either tall or semi-dwarf lines that carried *Rht-B1b, Rht-D1b,* or *Rht-8* to investigate how each semi-dwarfing allele impacts gluten composition and flour pasting properties. .

Findings: None of the semi-dwarfing alleles impacted starch properties. Each reduced flour protein content compared to the tall variety with the largest decreases in *Rht-B1b* (1.8%) and *Rht-D1b* (1.5%). The semi-dwarfing lines increased the gluten index (21.5%) compared to *Rht-1a*. Using SE-HPLC we determined that the semi-dwarfing lines had an increased relative abundance of high molecular weight glutenins compared to the tall variety.

Conclusions: This study indicates that the *Rht-1* semi-dwarfing alleles increase dough mixing time and tolerance by increasing the relative abundance of high molecular weight glutenins yielding stronger dough.

Significance and Novelty: The semi-dwarfing alleles developed primarily for agronomic purposes have significant impacts on gluten index and starch swelling power.

KEYWORDS: gluten, *Rht-1*, semi-dwarf, starch, wheat

1. INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most important crops for human consumption. Its popularity is driven by its ability to grow well within a wide range of environments, as well as the products derived from wheat dough. Specifically, the extensibility and elasticity unique to wheat dough which allows gas bubbles to be trapped during the baking process has contributed to the global success of wheat. Both extensibility and elasticity play important roles in dough development. Breadmaking doughs are typically stronger and more elastic compared to pastry doughs which have lower protein content and greater extensibility. Together, extensibility and elasticity are referred to as dough viscoelasticity and grain storage proteins are the major determinant of the viscoelasticity of dough (Shewry, Halford, Belton, & Tatham, 2002). Wheat grain protein accounts for approximately 8 - 18% of grain dry weight (Shewry et al., 2009). The major class of storage protein in wheat grain is gluten (Shewry, et al., 2002). Gluten is composed of hundreds of different protein subunits which form a complex matrix in dough (Wrigley & Bietz, 1988: reviewed in Wieser, 2007). Gluten can be separated into its two most prevalent fractions based on their solubility; the alcohol soluble gliadins, and the alcohol insoluble

glutenins. Gliadins are considered less elastic than glutenins and are primarily responsible for the extensibility of the dough. The glutenins are responsible for dough elasticity and cohesion which determines dough strength (Weiser, 2007). Furthermore, insoluble polymeric proteins are positively correlated with bake mixing time, whereas soluble polymeric proteins are negatively correlated with bake mixing time (Park, Bean, Chung, & Seib, 2006).

Glutenins are further characterized by their molecular weight. Low molecular weight glutenins account for approximately 20% of gluten proteins (Weiser & Kieffer 2001). High molecular weight glutenins account for 10% of gluten proteins and have a molecular weight ranging from 67 – 83 kDa (Wieser, 2007). Of gluten component proteins, high molecular weight glutenins have the greatest impact on dough strength (Wrigley, Bekes, & Bushnuk, 2006; Weiser & Kieffer 2001). High molecular weight glutenins are largely controlled by loci found on the group 1 chromosomes, *Glu-A1*, *Glu-B1*, and *Glu-D1* (Payne, 1987). Recent studies have shown that these genes, as well as those associated with gliadins, are regulated by both *cis* and *trans* loci (Plessis, Ravel, Bordes, Balfourier, & Martre, 2013). These transcription factors have been shown to impact the quantity and composition of glutenins and gliadins (Plessis et al., 2013).

In addition to grain protein, starch is also important in dough rheology. Starch accounts for 65-73% of the dry weight of flour (Pomeranz, 1988). It plays a key role in water absorption and interacts directly with the gluten matrix (Sandstedt, 1961, 1955; Petrofsky & Hoseney, 1995). Furthermore, the rate of starch gelatinization directly impacts dough expansion during baking (Kusunose, Fugii, & Matsumoto, 1999).

Starch is composed of large A-type and small B-type granules. The A-type granules have a diameter greater than 10 μm and are more disk shaped than spherical; B-type granules have a diameter smaller than 10 μm and are spherical (Soulaka & Morrison, 1985; Vermeylen, Goderis, Reynaers & Delcour, 2005; Kim & Huber, 2008). Although A-type granules account for greater than 70% of total starch weight, 90% of granules are B-type (Bechtel, Zayas, Kaleikau, & Pomeranz, 1990; Raeker, Gaines, Finney, & Donelson, 1998; Peng, Gao, Abdel-Aal, Hucl, & Chibbar, 1999). The surface of these granules has a direct impact on dough rheology (Sipes, 1993) and the ratio of A- and B-type granules impacts bread making (Park, Chung, & Seib, 2005).

Grain protein and starch content are quantitative traits controlled by many genes and influenced by the environment. One gene that has large impacts on protein content is *Reduced*

Height (*Rht-1*). There is a *Rht-1* gene on each of the group 4 chromosomes (Gale, Law, & Worland, 1975; Gale & Marshall, 1975, 1976; McVittie, Gale, Marshall, & Westcott, 1978; Sourdille et al., 1998). Dominant acting mutant forms of *Rht-1* reduce plant height and increase yield. The two most prevalent semi-dwarfing mutations are found in the B and D genome, *Rht-B1b* and *Rht-D1b*, respectively. Both alleles contain a premature stop codon near the RHT protein N terminus (Peng, Richards, & Hartley, 1999). The resultant truncated protein partially inhibits the plant's ability to respond to gibberellic acid (GA) (Allan, Vogel, & Craddock, 1959). The agronomic result is a 20% height reduction, increased productive tillers, and a 10% increase in grain yield (Flintham, Börner, Worland, & Gale, 1997). *Rht-B1b* and *Rht-D1b* are also associated with decreased kernel size and grain protein content (Flintham et al., 1997; Gooding, Cannon, Thompson, & Davies, 1999; Lanning et al., 2012; Mann et al., 2009). *Rht-8* is another gene which reduces plant height but does not interfere with the plant's ability to perceive gibberellic acid (Korzun, Röder, Ganal, Worland & Law, 1998; Rebetzke & Richards, 2000). *Rht-8* reduces plant height approximately 6.5% by impacting the plant's ability to respond to brassinosteroids (Lanning et al., 2012; Gasperini, Greenland, Hedden, Dreos, Harwood, & Griffiths, 2012). *Rht-8* was included as part of this study to ensure that the impact of *Rht-B1b/Rht-D1b* was due to the *Rht-1* mutations, and not semi-dwarfed plant architecture.

Limited work has been done to investigate the impact of the *Rht-1* semi-dwarfing alleles on bread making and end use quality. Sherman et al. (2014) associated *Rht-D1b* with decreased flour yield, protein, and loaf volume, but increased mixing tolerance and bake mixing time. We used near isogenic lines carrying either *Rht-B1b, Rht-D1b*, or no semi-dwarfing mutation to evaluate the impact of the semi-dwarfing alleles on end use quality (Jobson, Martin, Schneider, & Giroux, 2018). We also observed a decrease in flour protein content (1.8% *Rht-B1b*, 1.5% *Rht-D1b*), but an increase in mixograph mixing time (1.8 minutes) and tolerance when compared to *Rht-1a*.

The purpose of this study was to investigate the impact of the *Rht-1* semi-dwarfing alleles on dough strength and grain composition to better understand how they increase dough strength despite reducing flour protein content. For this study we used near isogenic lines developed in a tall hard red spring wheat cultivar. Lines either carried *Rht-B1b*, *Rht-D1b*, no semi-dwarfing gene (*Rht-1a*), or *Rht-8*.

The *Rht-1* semi-dwarfing alleles have been incorporated into most modern wheat cultivars. Therefore, it is important to not only understand their agronomic impact, but also their impact on end use quality and bread making. This study provides new insight into the impact of the semi-dwarfing alleles on grain protein and starch composition in relation to bread making quality. The semi-dwarfing alleles are some of the most broadly used genes in wheat breeding programs. Although there has been extensive research regarding their impact on plant growth and development, there is very limited research regarding their impact on bread making and end use quality. This study provides a comprehensive analysis of the impact of the semi-dwarfing alleles on starch and protein in relation to bread making; and illustrates how genes which significantly impact agronomic traits also influence product quality.

2. MATERIALS AND METHODS

2.1. Plant Material

This study used near isogenic lines (NILs) which carried either no semi-dwarfing alleles, *Rht-B1b, Rht-D1b,* or *Rht-8*. The NILs were developed in the standard height, hard red spring wheat, "Fortuna" (CI 13596) as described by Lanning et al. (2012). "Hi-Line" (PI 549275) was the donor parent for the *Rht-B1b* allele, "McNeal" (PI 574642) served as the donor of the *Rht-D1b* allele, and "Mara" (PI 244854) was the donor of the *Rht-8* allele. All lines were backcrossed to Fortuna as the recurrent parent to the BC_4 generation. The genotype of each line was confirmed in the BC_4 generation using the markers described by Ellis, Spielmeyer, Gale, Rebetzke, & Richrds (2002) and Ellis, Rebetzke, Azanza, Richards, & Spielmeyer (2005).

2.2. Field Design and Conditions

Trials for this study were grown as described in Jobson et al., (2018) in 2017 at the Arthur H. Post Field Research Center near Bozeman, MT (latitude 45.6 N, longitude 111.00 W, elevation 1,455 m, soil type: Amsterdam silt loam). Plants were grown under both irrigated and rainfed conditions. The trials received 13.8 cm of precipitation throughout the growing season with the irrigated field receiving an additional 10.2 cm of water with half supplied one week prior to and half one-week post heading.

146 The NILs (*Rht-B1b, Rht-D1b, Rht-8*, and *Rht-1a*) were grown in a randomized complete
147 block design with five replications in both the irrigated and rainfed trials. The plots were 3 m
148 long and 4 rows wide, with 30 cm between rows. Seeds were planted at a rate of 3.3 g per m of
149 row.

150 **2.3. Milling**

151 Milling and quality analysis were done according to AACC approved methods (American
152 Association of Cereal Chemists, 2000). Samples were cleaned using a Forster Cyclone Grain
153 Scourer, 1930, size 6 (Forster Manufacturing CO, Wichita, KS
154). Dockages were removed using a Dockage Test Machine XT7 2014 (Carter International,
155 Minneapolis, MN). Samples were tempered to 14.5 % moisture (AACC Method 26-10.02) and
156 milled into straight grade white flour and bran fractions using a Quadrumat Jr II Mill (C.W.
157 Brabender Instruments Inc., Hackensack, NJ). The milled samples were further cleaned using a
158 149 µm USA Standard Testing Sieve (Seedburo Equipment CO., Chicago, IL) and a Ro-Tap
159 RX-29 shaker (W.S. Tyler, Mentor, OH).

160 **2.4. Flour Properties**

161 Flour protein and moisture content were measured using a Foss Infratec 1241 Machine
162 (Foss Analytics, Eden Prairie, MN; AACC Method 39-11.01). Whole wheat flour for flour
163 swelling power analysis and starch swelling power was milled using a Laboratory Mill 3303
164 (Perten, Springfield, IL). Starch was extracted and purified from 300 mg of whole wheat flour as
165 described by Hogg *et al*. (2013). Flour swelling power was measured according to AACC
166 Method 56-21.01. Gluten Index was measured using the Glutomatic System (Perten, Springfield,
167 IL; AACC Method 38-12.02). The pasting property of flour was measured using a Perten Rapid
168 Visco Analyser 4500 (Perten; AACC Method 76-21.02).

169 **2.5. Starch Granule Visualization**

170 Measurements of starch granules were done using images captured using a Zeiss Supra
171 55 VP field emission gun-scanning electron microscope, (Carl Zeiss Microscopy, Peabody, MA).
172 Three starch samples purified from flour from each genotype grown under irrigated conditions
173 were imaged. Each sample was imaged 5 times. Measurements of A- and B-type granules were
174 determined by measuring the maximal diameter of three A- and three B-type granules from each
175 image; totaling 15 measurements for each of the three biological replicates.

176 **2.6. Protein molecular weight distribution**

Flour from 3 replications of each genotype grown under irrigated conditions was analyzed at the USDA-ARS Hard Spring and Durum Wheat Quality Laboratory at Fargo, North Dakota for protein molecular weight distribution analysis. Protein molecular weight distribution (MWD) parameters were measured using size exclusion high performance liquid chromatography (SE-HPLC) as described by Gupta, Khan, & MacRitchie (1993) and Ohm, Hareland, Simsek, & Seabourn (2009). The extractable and unextractable protein fractions were obtained from 10mg (14% moisture) of flour using a sodium phosphate/ sodium dodecyl sulfate (SDS) solution (0.5% SDS and 0.5 M sodium phosphate, pH 6.9). The SDS extractable protein fraction was then solubilized in 1 ml of a buffer solution, and vortexed for 5 minutes at 2,000 rpm using a vortex mixer (Pulsing Vortex Mixer; Fisher Scientific, Hampton NH). The extractable protein fraction was then separated by centrifugation at 20,000 g (Eppendorf Centrifuge 5424, Hamburg, Germany) and filtered through a 0.45 μm polyvinylidene difluoride syringe filter. The unextractable protein fraction was solubilized by sonicating the residue in 1 mL of the buffer solution for 30 sec (Sonic Dismembrator 100; Fisher Scientific). The unextractable protein fraction was then separated using centrifugation and filtration as described for the extractable fraction. Immediately after filtration both the SDS extractable and unextractable protein fractions were heated at 80 °C for 2 min to prevent protein hydrolysis. Ten μL of each fraction was then injected individually for SE-HPLC fractionation.

Size exclusion HPLC was done using a liquid chromatograph (Agilent 1100; Agilent Technologies, Santa Clara, CA) loaded with a size exclusion narrow bore column (300 × 4.6 mm, Yarra 3 um SEC SEC-4000; Phenomenex, Torrance, CA) and a guard cartridge (BIOSEP SEC S4000; Phenomenex). The SE-HPLC system was run at a flow rate of 0.5 mL/min using an isocratic mobile phase of 50% acetonitrile and 0.1% (v/v) trifluoroacetic acid aqueous solution. Absorbance data were attained at 214 nm by a photodiode array detector (Agilent 1200; Agilent Technologies, Santa Clara, CA). UV absorbance data were analyzed by in-house programs coded using MATLAB software (MathWorks, Natick, MA) as described by Ohm, Hareland, Simsek, & Seabourn et al. (2009). Size exclusion HPLC profiles were divided into four fractions (F) as follows, F1: 3.5– 4.7 min, F2: 4.7–5.2 min, F3: 5.2-5.8 and F4: 5.8-7.4 min. Size exclusion HPLC fractions (F1–4) were reported to be composed primarily of polymeric proteins for F1 and F2, gliadins for F3, and albumin and globulins for F4 (Larroque, Gianibelli, Batey, &

MacRitchie, 1997; Malalgoda, Ohm, Meinhardt, & Simsek, 2018). The protein molecular weight distribution parameters were derived from UV absorbance data from the four fractions.

2.7. RNA Sequencing

Grain used for expression analysis was collected and immediately frozen using liquid nitrogen at 21 days past anthesis. The frozen grain was ground using a mortar and pestle, and total RNA was extracted using a RNeasy Plant Mini Kit (Qiagen, Valencia, CA) (Oiestad et al., 2016). The extracted RNA was quantified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). ArrayStar (DNASTAR, Madison, WI) was used to analyze the sequence data. The parameters were match setting at 90% for a minimum of 100 bp. The data are reported as reads per kilobase of transcript for million mapped reads (RPKM) (Mortazavi, Williams, McCue, Schaeffer, & Wold, 2008). The data was initially analyzed globally using the most recent wheat genome sequence (Appels, et al., 2018). We then performed a targeted expression analysis focused on genes previously identified to be involved in grain starch (Cao, Hu, & Wang, 2012) and protein synthesis (Kawaura, Mochida, & Ogihara, 2005). The data were normalized to *Act-2*. *Act-2* encodes an actin-like protein and has been previously shown to be a reliably expressed gene for RNAseq dataset normalization (Tenea, Peres, Cordeiro, & Maquet, 2011).

2.8. Statistical Analysis

Response variables where data were obtained on all replications were analyzed using an analysis of variance model for a randomized complete block design which combined both rainfed and irrigated environments. All factors were considered fixed. A model for a completely randomized design was used for protein molecular weight distribution and starch granule size variables where data were collected on a subset of the replications. The Least Significant Difference (LSD) value to compare differences between genotypes was calculated following a significant F ratio ($P<0.05$) using the "Agricolae" R v1.3.3 package (De Mendiburu & Simon, 2015). The *P* value presented for the expression analysis was calculated using a two-tailed, independent sample *t*-test. This value represents any variance in expression between the wildtype and *Rht-B1b* lines.

3. RESULTS

3.1. Flour and Starch Pasting Properties

We observed the expected reduction in flour protein in the semi-dwarf lines compared to the tall *Rht-1a*. *Rht-B1b* and *Rht-D1b* decreased flour protein content by 1.8% and 1.5% compared to *Rht-1a* ($P<0.05$; Table 1). *Rht-B1b* and *Rht-D1b* increased gluten index 21.5% compared to *Rht-1a* (95 % vs 74 %) (*P<0.05*; Table 1*)*. *Rht-8* had intermediate flour protein content and gluten index between the semi-dwarf and tall varieties. *Rht-B1b* and *Rht-D1b* had no measurable impact compared to *Rht-1a* on starch swelling power (Table 1), flour swelling power, or starch granule size (data not shown).

The semi-dwarfing alleles had an impact on the pasting properties of the flour measured using RVA. The peak viscosity value was increased 20% in the semi-dwarfing lines compared to *Rht-1a* (data not shown) and time to peak was increased slightly. The total setback value was also increased in *Rht-B1b* (26%) and *Rht-D1b* (22%) compared to *Rht-1a* (data not shown)

3.2. Protein Molecular Weight Distribution

SE-HPLC results are divided by those proteins which were SDS-Extractable, and those which were not. The first two parameters in each group (UP1, UP2, EP1, EP2) represent the proportion of polymeric glutenin proteins in total proteins. Specifically, UP1 and UP2 are associated with the large molecular weight glutenins. We observed an increase in UP1 and UP2 in *Rht-B1b* (UP1: 14.92%, UP2: 4.39%) and *Rht-D1b* (UP1: 14.65%, UP2: 4.33%) compared to *Rht-1a* (UP1: 12.73%, UP2: 3.80%) (*P<0.05;* Table 2). The third fraction is associated with the unextractable and extractable gliadin subunits (UP3 and EP3). The EP3 value was decreased in the semi-dwarfing lines (*Rht-B1b*:32.7%, *Rht-D1b*:33.42%) compared to the tall variety (35.84%) (*P<0.05*; Table 2). There was no measurable difference between genotypes for the unextractable gliadins. The fourth fraction (UP4 and EP4) is associated with the albumins and globulins, we did not detect any significant differences between genotypes for this fraction.

3.3. RNA Sequencing

RNA sequencing data was initially analyzed globally. We also performed a targeted analysis focused on genes associated with seed storage proteins (Table 3). We found no statistically significant differences in storage protein gene expression between *Rht-B1b* and *Rht-1a*.

DISCUSSION

Rht-B1b and *Rht-D1b* have a significant impact on many aspects of wheat plant growth and development. Because they reliably decrease plant height and increase yield, they are now

present in most modern wheat varieties. However, despite their agronomic importance, there is limited research regarding their impact on protein and starch in relation to bread making. Since the ability of wheat flour to be baked into bread is one of the primary reasons for the global success of wheat, it is important to understand how yield genes, such as *Rht-1*, impact end use quality and bread making.

A previous study (Jobson et al., 2018) showed that *Rht-B1b* and *Rht-D1b* increase dough mixing time (4.6 minutes) while reducing flour protein. In this study we evaluated the impact of *Rht-B1b* and *Rht-D1b* on dough gluten index, starch properties, and storage protein composition to understand how *Rht-B1b* and *Rht-D1b* impact dough mixing properties. *Rht-B1b* and *Rht-D1b* did not impact flour or starch swelling power, or starch granule size. Our previous study also showed *Rht-B1b* and *Rht-D1b* had no impact on alpha amylase activity (Jobson et al., 2018). Based on these results, it is unlikely that the semi-dwarfing alleles significantly impact starch content or composition.

There were measurable differences in pasting viscosity between *Rht-B1b/Rht-D1b* and *Rht-1a*. Semi-dwarf NILs flour had increased final viscosity as well as peak time compared to the tall NIL flour. This may be explained by previous findings which describe an inverse relationship between flour protein content and final viscosity and total setback (Lee, 2016; Katyal et al., 2018).

Despite having a lower flour protein, *Rht-B1b* and *Rht-D1b* increased gluten index 21.5% compared to *Rht-1a* (95% vs 74%). This agrees with our previous study which illustrated that despite decreasing total protein content, the *Rht-B1b* and *Rht-D1b* semi-dwarfing alleles increase dough strength (Jobson et al., 2018). This may be explained by a difference in the abundance of major storage proteins. Barak, Mudgil, and Khatkar (2014) and Dhaka and Khatkar (2015) found that an increased ratio of gliadins to glutenins decreased gluten index and dough stability. Barak et al. (2014) added fractionated glutenins and gliadins to fortified flour in increments of 2, 4, 6, 8, and 10%. The addition of gliadins decreased the stability and mixing time of the dough, while the addition of glutenins increased the dough stability and mixing time. A 2% addition of glutenins resulted in a 100% increase in the dough stability. Based on SE-HPLC data, *Rht-B1b/Rht-D1b* have a positive impact on glutenin percentage, specifically the high molecular weight SDS unextractable polymers. Previous SE-HPLC studies have shown that these high molecular weight SDS unextractable protein polymers have a significant impact on increasing

dough strength, which may partially explain the increased dough strength associated with the semi-dwarfing alleles (Tsilo, Mudgil, & Khatkar, 2010; Dachkevitch & Autran 1989; Singh, Donovan, & MacRitchie, 1990; Bangur, Batey, McKenzie, & MacRitchie, 1997; Park et al., 2006).

For almost all traits, *Rht-8* was intermediary between *Rht-B1b/Rht-D1b* and *Rht-1a*. This may be due to its intermediate grain protein content. However, further research is needed to understand the mechanism behind the impact of *Rht-8* on grain composition and end use quality.

CONCLUSIONS

This study provides a comprehensive analysis of the impact of the *Rht-B1b* and *Rht-D1b* semi-dwarfing alleles on flour protein composition, starch and flour pasting properties. We found that despite decreasing total flour protein content, *Rht-B1b* and *Rht-D1b* increase dough strength compared to the tall NIL by altering the composition of gluten component storage proteins. We observed that *Rht-B1b* and *Rht-D1b* increase the relative abundance of glutenins compared to gliadins, which has previously been shown to increase dough strength. Further studies will be needed to determine how the semi-dwarfing alleles alter gluten storage protein composition.

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Tables:

Table I: Impact of *Rht* semi-dwarfing alleles on gluten index, starch swelling, and flour viscosity.

	Flour Protein (%)	Gluten Index (%)	Starch Swelling Power (g/g)[a]	RVA[b] Final Viscosity[c]	RVA Peak Time (min)
Rht-1a	15.0 ± 0.13[a]	74 ± 0.03[c]	23.4 ± 3.31[a]	159.5 ± 6.8[b]	15.8 ± 0.09[c]
Rht-8	14.6 ± 0.19[b]	82 ± 0.03[b]	20.28 ± 2.42[a]	168.1 ± 11.2[b]	15.9 ± 0.13[bc]
Rht-B1b	13.2 ± 0.05[d]	96 ± 0.03[a]	21.3 ± 5.42[a]	199.1 ± 35.7[a]	16.0 ± 0.09[ab]
Rht-D1b	13.5 ± 0.11[c]	95 ± 0.02[a]	21.9 ± 2.94[a]	195.2 ± 13.7[a]	16.03 ± 0.19[a]
LSD (0.05)	0.16	2.36	*N.S.*	16.9	0.08

[a] Starch swelling power reported as grams of water absorbed/grams of starch.

[b] RVA: Rapid Visco Analyser

[c] Final Viscosity measured in Rapid Visco Units.

N.S. no significant difference between groups.

Values represent the mean of combined rainfed and irrigated plots ± standard error.

Means followed by different letters within the same column are statistically different ($P \leq 0.05$).

$n = 10$, where n represents one plot in either rainfed or irrigated conditions.

Flour protein previously reported in Jobson et al. (2018).

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568 **Table II:** Impact of Rht-1 semi-dwarfing alleles upon wheat flour storage protein distribution.

SDS Extractable	Mean % Area
EP1	
Rht-1a	15.63 ± 0.08[a]
Rht-8	14.96 ± 0.26[b]
Rht-B1b	15.13 ± 0.34[ab]
Rht-D1b	15.18 ± 0.38[ab]
LSD	0.54
EP2	
Rht-1a	7.60 ± 0.25[a]
Rht-8	7.46 ± 0.17[a]
Rht-B1b	7.49 ± 0.21[a]
Rht-D1b	7.45 ± 0.17[a]
LSD	0.38
EP3	
Rht-1a	35.84 ± 0.35[a]
Rht-8	35.18 ± 0.12[b]
Rht-B1b	32.70 ± 0.18[c]
Rht-D1b	33.42 ± 0.20[d]
LSD	0.42
EP4	
Rht-1a	16.34 ± 0.65[a]
Rht-8	16.86 ± 0.68[a]
Rht-B1b	17.04 ± 0.48[a]
Rht-D1b	17.00 ± 0.54[a]
LSD	1.12
SDS Unextractable	
UP1	
Rht-1a	12.73 ± 0.64[c]
Rht-8	13.54 ± 0.68[bc]
Rht-B1b	14.92 ± 0.48[a]
Rht-D1b	14.65 ± 0.59[ab]

LSD	1.14
UP2	
Rht-1a	3.80 ± 0.07[c]
Rht-8	4.06 ± 0.06[b]
Rht-B1b	4.39 ± 0.14[a]
Rht-D1b	4.33 ± 0.07[a]
LSD	0.17
UP3	
Rht-1a	5.21 ± 0.57[a]
Rht-8	5.04 ± 0.11[a]
Rht-B1b	5.26 ± 0.32[a]
Rht-D1b	5.02 ± 0.10[a]
LSD	0.42
UP4	
Rht-1a	3.07 ± 0.04[b]
Rht-8	2.95 ± 0.09[ab]
Rht-B1b	2.91 ± 0.11[a]
Rht-D1b	2.84 ± 0.13[ab]
LSD	0.19

Values represent the mean of irrigated plots ± standard deviation, n=3.

Means followed by different letters within the same column are statistically different ($P \leq 0.05$).

EP: SDS Extractable proteins, higher content of low molecular weight subunits.

UP: SDS Unextractable proteins, higher content of high molecular weight subunits.

EP1, EP2, UP1, and UP2 primarily represent the polymeric proteins, EP3/UP3 represent the gliadins, and EP4/UP4 represent albumins and globulins (Larroque et al., 1997; Malalgoda et al., 2018).

Table III: Expression of wheat seed storage protein genes in developing grains 21 days past anthesis. Data is reported as the average reads per kilobase million (RPKM), n=3 individual plants grown under irrigated conditions, *P*-value represents a two tailed independent *t* test, all expression values were normalized to actin (Tenea et al., 2011).

	Protein Type	**Accession #**	***Rht-B1b* Average RPKM**	***Rht-1a* Average RPKM**	***P*-value**	***Rht-B1b/ Rht-1a***
Gliadins	alpha gliadin	U51306	12353	22366	0.28	0.55
	alpha/beta gliadin	M11075	52764	58102	0.54	0.91
	gamma gliadin	M16064	79750	69377	0.68	1.15
	omega gliadin	AF280605	6708	9290	0.36	0.72
Glutenins	HMW x-type Bx7	DQ119142	16179	15119	0.82	1.07
	HMW x-type 1Dx5	X12928	9380	8377	0.70	1.12
	HMW y-type 1Dy	X03041	9250	10509	0.17	0.88
Control	Actin	AB181991	353	353		