GENETIC OPTIMIZATION OF SEED SIZE, PLANT HEIGHT, AND TILLERING TRAITS FOR ENHANCED YIELD IN MONTANA WHEAT

by

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DEDICATION

This work is dedicated to my late grandma, Joyce E. Lagerlund. She played a huge role in running the farm on which my mother grew up, and dedicated a lifetime to supporting her family, farm workers, and the farming community in general. She was a source of constant encouragement to me personally, and her legacy will continue to inspire me as I pursue a career in support of agriculture.

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ABSTRACT

Wheat (*Triticum* spp.) production is central to Montana State's economy. In 2024, 5.26 million acres of wheat were planted in Montana, accounting for 11.4 % of the United States' total planted acreage. In 2024, nearly half of Montana's wheat acreage was planted with varieties developed by breeding programs at Montana State University. This thesis research directly supports these breeding programs to develop superior wheat varieties for Montana farmers. Natural alleles of *Grain Number Increase-1 (GNI-A1)* in spring wheat (*Triticum aestivum* L.) were studied in field experiments to examine their impact on seed size, yield, and protein content. The alleles *GNI-A1*-105K and *GNI-A1*-105Y increase seed number without reducing grain quality. Ethyl methanesulfonate (EMS) derived mutant alleles of the Green Revolution gene *Reduced Height-1 (Rht-1)* were examined in durum wheat (*Triticum turgidum* L. subsp. *durum*) and in spring wheat to show how manipulating plant height can improve yield and other traits. Specifically, the alleles *Rht-B1b*-E529K and *Rht-A1*-E63K impart an intermediate height phenotype, improving seedling emergence and grain protein content, and increasing grain yields in some environments. Finally, natural and EMS derived mutant alleles of *Teosinte Branched-1 (TB1)* were tested in durum wheat, spring wheat, forage winter wheat (*Triticum aestivum* L.), and triticale (*Triticosecale* spp.) to develop lines with higher tillering potential, and consequently higher grain yields and forage biomass. Introducing one or two nonsense alleles of *TB1* into polyploid wheat crops reduces *TB1* function, increasing tiller number and tillering plasticity, ultimately improving yields in certain environments. Research on these genes has led to the development of superior Montana adapted germplasm that will help breeding programs to accelerate gains in yield while maintaining high cereal quality. In addition, this research provides wheat breeders around the world with genetic stock and background data they can use to optimize seed size, height, and tillering potential according to their specific goals.

CHAPTER ONE

INTRODUCTION

Wheat Breeding in Montana

Wheat (*Triticum* spp.) production in Montana accounted for 8.7 % of total United States production (11.4 % of total planted acreage). U.S. production accounted for about 6 % of the global total (USDA, 2024b; USDA, 2024c). In 2024, Montana State University (MSU) varieties accounted for 50.3 % of winter wheat (*Triticum aestivum* L.) acreage planted in Montana, and 52.6 % of spring wheat (*Triticum aestivum* L.) acreage planted (USDA, 2024a). No MSU durum wheat (*Triticum turgidum* L. subsp. *durum*) varieties were planted as production acres, but with the recent releases of 'MT Raska' (PI 703025) and 'MT Blackbeard' (PI 703026), MSU variety durum acreage is expected to increase (Hogg et al., 2025). Altogether, at least 43 % of Montana wheat produced, 3.7 % of United States national wheat produced, and 0.22 %—or one in every 455 bushels—of global wheat produced was grown using varieties developed at MSU. These varieties are bred, tested, and released by MSU wheat breeding programs in the Plant Sciences and Plant Pathology Department in collaboration with the Department of Research Centers and the MSU Foundation Seed Program. These varieties are bred to enable high yield potential and to maintain high grain quality in harsh, dryland, Northern Great Plains environments. They address specific challenges that Montana farmers face including drought, diseases, pests, and maintenance of adequate test weight and grain protein content.

Natural and Artificial Allelic Diversity

Plant breeding requires continuous integration and maintenance of genetically diverse material to sustain forward progress. The underlying basis for this diversity is allelic variation—new combinations of different alleles that confer new phenotypes. This variation in alleles primarily is maintained through introduction of novel sources of germplasm from germplasm collections and other breeding programs, but it can also be induced artificially. Natural alleles originate from spontaneous mutations in the ancestors of modern crops. These mutations persisted through evolution and end up in modern varieties through germplasm introductions. Artificial alleles are ones that are altered by scientists through mutagenesis, or through gene editing and transgenics using modern biotechnological tools like CRISPR-Cas9 and agrobacterium mediated transformation (Doudna & Charpentier, 2014; Oladosu et al., 2016).

This research examines variability in naturally occurring alleles, and in artificial alleles created through mutagenesis. Natural allelic variation provides the basis for many of the traits present in modern food crops. One example is the difference between red and white grapes, caused by mutations in grapevine (*Vitis vinifera* L.) genes *VvMYBA1* and *VvMYBA2.* These mutant alleles likely originated from an ancient common ancestor that experienced a mutation event which resulted in expression of a desirable white grape phenotype. Progeny of this plant were eventually selected and propagated by ancient farmers and winemakers (Walker et al., 2007). Additionally, many useful traits have been developed in modern crop varieties through creation and integration of mutagenesis derived alleles (Maluszynski et al., 2000; Sikora et al., 2011). The grapefruit *(Citrus* × *paradisi)* variety ‘Rio Red’ is the most common grapefruit variety grown in Texas. Rio Red is characterized by its deep red flesh color, the result of a

mutation derived from exposure to thermal neutron radiation (Da Graça et al., 2004; Hensz, 1985). Likewise, the semidwarf trait in 'Calrose 76' rice (*Oryza sativa* L.) was generated by exposing the variety 'Calrose' to Cobalt-60 gamma radiation, inducing a loss of function mutation in the gene *SD1.* Numerous modern rice varieties containing this semidwarf trait are descended from Calrose 76 (Rutger, 2009; Rutger et al., 1977). Italian durum wheat varieties with improved lodging resistance and increased grain yield were developed in the 1960s and 1970s via exposure to thermal neutron, fast neutron, and x-ray radiation. Many durum wheat varieties grown in Europe today have traits originally inherited from these mutagenized lines. (De Vita & Taranto, 2019; Scarascia Mugnozza, 2005). These mutagenesis techniques do not fall under regulations that cover more modern transgenic and gene editing techniques. As such, traditional mutagenesis methods are deemed safe to use in the development of crop varieties grown in both conventional and organic systems, without additional regulation beyond that of other traditional plant breeding methods.

Ethyl methanesulfonate (EMS) is a chemical commonly utilized to induce plant mutagenesis and create novel allelic diversity. When seeds are exposed to EMS solution, guanine residues in the DNA of the seeds' embryos are alkylated. This causes mispairing of amino acids resulting in G:C to A:T transition mutations (Greene et al., 2003). EMS was first used as a mutagenesis tool to study fruit flies (*Drosophila* spp.) in the mid 1960's (Alderson, 1965). Since then, it has been used to induce heritable mutations in populations of many different crop species. These populations can be utilized in forward genetics research approaches in which a novel phenotype within a mutagenized population is selected, and the causal mutation within that line is then identified and characterized. These populations can also be used in reverse genetics

research approaches in which mutations in a gene of interest are first identified within a mutagenized population, and lines carrying that mutation are tested experimentally to characterize the effect of that novel mutation (Jankowicz-Cieslak & Till, 2016). Modern genotyping methods enable sequencing of the protein coding regions in EMS mutagenized populations followed by the subsequent alignment and cataloging of those sequences. This allows plant breeders to search databases for a specific mutation of interest, and to obtain the catalogued line containing that desired mutation. This method is known as Targeting Induced Local Lesions in Genomes or TILLING and is a highly effective method of utilizing reverse genetics to search for potentially beneficial mutant alleles (Colbert et al., 2001; McCallum et al., 2000). EMS mutagenesis induced alleles in this study are derived from publicly available TILLING populations as well as from EMS mutagenized populations developed at Montana State University (Feiz et al., 2009; Krasileva et al., 2017).

Genes of Interest

This research examines allelic variation in specific genes that have the potential to accelerate development of superior wheat varieties for Montana producers. Three previously characterized genes in wheat were examined to identify novel alleles that can improve crop phenotype. *Grain Number Increase-1 (GNI-1)* affects floret fertility and grain size, *Reduced height-1 (Rht-1)* affects plant height, and *Teosinte Branched-1 (TB1)* affects branching and spike morphology (Dixon et al., 2018; Pearce et al., 2011; Sakuma et al., 2019; Van De Velde et al., 2021).

Floret fertility determines seed set in a wheat spike. Higher fertility increases the number of seeds per spike, but seeds are generally smaller due to environmental and physiological

limitations. *GNI-1* is a HOX-1 class transcription factor that promotes floret abortion. Lower expression or function of this gene is associated with higher floret fertility, more seeds per spike, and oftentimes higher grain yield in modern varieties with optimized physiological resource allocation (Golan et al., 2019; Sakuma et al., 2019). However, smaller seeds can also be associated with decreased grain quality (Ficco et al., 2020). Field experiments are conducted on recombinant inbred line population (RIL) and heterogenous inbred family (HIF) derived near isogenic line (NIL) populations that varied for different alleles of *GNI-A1.* The effect of naturally occurring mutant alleles on grain quality is further characterized in post harvest milling experiments.

Rht-1 is a DELLA protein encoding gene that is a key part of the gibberellic acid signaling pathway (Pearce et al., 2011; Van De Velde et al., 2021). Reduction in wheat plant height through semidwarfing alleles *Rht-B1b* and *Rht-D1b* characterized the Green Revolution of the mid 20th century. Semidwarf varieties did not lodge when farmers used modern fertilizers and pesticides to produce more vigorous crops, drastically increasing global food production (Hedden, 2003; Hoogendoorn et al., 1990). However, these alleles have drawbacks, including reduced seedling emergence, reduced protein content, and reduced yields in extreme drought environments (Mathews et al., 2006; Schillinger et al., 1998). Field and lab experiments are conducted using NIL populations in spring wheat and in durum wheat to search for alleles that induce intermediate height, between traditional semidwarf and tall lines. Intermediate height alleles are characterized to determine how they affect protein function, grain size, grain protein content, grain yield, and seedling emergence.

TB1 is a TCP class transcription factor gene that promotes apical dominance in plants (Martín-Trillo & Cubas, 2010). Manipulating function or expression of this gene alters plant morphology, creating taller single stalked plants or more highly branched plants (Aguilar-Martinez et al., 2007; Dixon et al., 2018; Doebley et al., 1997). Missense and nonsense mutant alleles of *TB1* are backcrossed into Montana adapted wheat varieties to increase productive tiller number and grain yield. Eleven location/years of field experiments are conducted to study EMS derived mutant durum wheat alleles across Montana. In spring wheat, the naturally occurring *TB-D1b* mutant allele is studied in RIL population experiments. Additionally, EMS derived mutant alleles of *TB1* are studied in spring wheat and winter wheat NIL population experiments. Similar projects have been initiated in in triticale *(Triticosecale spp.)*.

These studies employ EMS mutagenesis, sanger sequencing, protein interaction assays, milling quality analysis, and marker assisted selection in the lab; seedling emergence studies, RIL population development, and NIL population development in the greenhouse; plant phenotyping, seeding rate trials, and yield trials at research farms; and finally, data compilation, image analysis, statistical modeling, and data visualization on the computer. Results show which *GNI, Rht-1,* and *TB1* alleles breeders should integrate and select for to develop increasingly high yielding and high quality wheat varieties for Montana farmers.

References

Aguilar-Martinez, J. A., Poza-Carrion, C., & Cubas, P. (2007). Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. *Plant Cell*, *19*(2), 458-472. https://doi.org/10.1105/tpc.106.048934

Alderson, T. (1965). Chemically induced delayed germinal mutation in Drosophila. *Nature*, *207*(4993), 164-167. https://doi.org/10.1038/207164a0

Colbert, T., Till, B. J., Tompa, R., Reynolds, S., Steine, M. N., Yeung, A. T.,…Henikoff, S. (2001). High-throughput screening for induced point mutations. *Plant physiology*, *126*(2), 480-484. https://doi.org/10.1104/pp.126.2.480

Da Graça, J., Louzada, E., & Sauls, J. (2004). The origins of red pigmented grapefruits and the development of new varieties. Proceedings of the International Society of Citriculture,

De Vita, P., & Taranto, F. (2019). Durum wheat (Triticum turgidum ssp. durum) breeding to meet the challenge of climate change. In *Advances in Plant Breeding Strategies: Cereals: Volume 5* (pp. 471-524). Springer. https://doi.org/10.1007/978-3-030-23108-8_13

Dixon, L. E., Greenwood, J. R., Bencivenga, S., Zhang, P., Cockram, J., Mellers, G.,…Boden, S. A. (2018). TEOSINTE BRANCHED1 Regulates Inflorescence Architecture and Development in Bread Wheat (Triticum aestivum). *Plant Cell*, *30*(3), 563-581. https://doi.org/10.1105/tpc.17.00961

Doebley, J., Stec, A., & Hubbard, L. (1997). The evolution of apical dominance in maize. *Nature*, *386*(6624), 485-488. https://doi.org/10.1038/386485a0

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213), 1258096. https://doi.org/10.1126/science.1258096

Feiz, L., Martin, J., & Giroux, M. (2009). Creation and functional analysis of new Puroindoline alleles in Triticum aestivum. *Theoretical and Applied Genetics*, *118*(2), 247-257. https://doi.org/10.1007/s00122-008-0893-1

Ficco, D. B. M., Beleggia, R., Pecorella, I., Giovanniello, V., Frenda, A. S., & Vita, P. D. (2020). Relationship between Seed Morphological Traits and Ash and Mineral Distribution along the Kernel Using Debranning in Durum Wheats from Different Geographic Sites. *Foods*, *9*(11), 1523. https://doi.org/10.3390/foods9111523

Greene, E. A., Codomo, C. A., Taylor, N. E., Henikoff, J. G., Till, B. J., Reynolds, S. H.,…Odden, A. R. (2003). Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. *Genetics*, *164*(2), 731-740. https://doi.org/10.1093/genetics/164.2.731

Golan, G., Ayalon, I., Perry, A., Zimran, G., Ade-Ajayi, T., Mosquna, A.,…Peleg, Z. (2019). GNI-A1 mediates trade-off between grain number and grain weight in tetraploid wheat. *Theoretical and Applied Genetics*, *132*(8), 2353-2365. https://doi.org/10.1007/s00122-019-03358-5

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

Hensz, R. A. (1985). *'Rio Red,' a new grapefruit with a deep-red colour. Journal of the Rio Grande Valley Horticultural Society, 38*, 75–76.

Hogg, A. C., Hale, C. O., Tillett, B. J., Huang, L., Carr, P. M., Eberly, J.,…Lamb, P. (2025). Registration of 'MT Blackbeard'and 'MT Raska'durum wheat. *Journal of Plant Registrations*, *19*(1), e20425. https://doi.org/10.1002/plr2.20425

Hoogendoorn, J., Rickson, J. M., & Gale, M. D. (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

Jankowicz-Cieslak, J., & Till, B. J. (2016). Chemical mutagenesis of seed and vegetatively propagated plants using EMS. *Current protocols in plant biology*, *1*(4), 617-635. https://doi.org/10.1002/cppb.20040

Krasileva, K. V., Vasquez-Gross, H. A., Howell, T., Bailey, P., Paraiso, F., Clissold, L.,…Dubcovsky, J. (2017). Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(6), E913-E921. https://doi.org/10.1073/pnas.1619268114

McCallum, C. M., Comai, L., Greene, E. A., & Henikoff, S. (2000). Targeted screening for induced mutations. *Nature biotechnology*, *18*(4), 455-457. https://doi.org/10.1038/74542

Maluszynski, M., Nichterlein, K., Van Zanten, L., & Ahloowalia, B. (2000). Officially released mutant varieties-the FAO/IAEA Database. *Plant Cell, Tissue and Organ Culture*, *65*(3), 175-177.

Martín-Trillo, M., & Cubas, P. (2010). TCP genes: a family snapshot ten years later. *Trends in plant science*, *15*(1), 31-39.

Mathews, K. L., Chapman, S. C., Trethowan, R., Singh, R. P., Crossa, J., Pfeiffer, W.,…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

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A.,…Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology & Biotechnological Equipment*, *30*(1), 1-16. https://doi.org/10.1080/13102818.2015.1087333

Pearce, S., Saville, R., Vaughan, S. P., Chandler, P. M., Wilhelm, E. P., Sparks, C. A.,…Phillips, A. L. (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

Rutger, J. (2009). The induced sd1 mutant and other useful mutant genes in modern rice varieties. *Induced plant mutations in the genomics era. Rome: Food and Agriculture Organization of the United Nations*, 44-47.

Rutger, J. N., Peterson, M. L., & Hu, C. (1977). Registration of Calrose 76 rice 1 (Reg. no. 45). *Crop Science*, *17*(6), 978-978. https://doi.org/10.2135/cropsci1977.0011183X001700060047x

Sakuma, S., Golan, G., Guo, Z. F., Ogawa, T., Tagiri, A., Sugimoto, K.,…Komatsuda, T. (2019). Unleashing floret fertility in wheat through the mutation of a homeobox gene. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(11), 5182-5187. https://doi.org/10.1073/pnas.1815465116

Scarascia Mugnozza, G. T. (2005). The contribution of Italian wheat geneticists: from Nazareno Strampelli to Francesco D'Amato. *Rome: Accademia Nazionale delle Scienze*, 53-75.

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. http://dx.doi.org/10.2134/agronj1998.00021962009000050002x

Sikora, P., Chawade, A., Larsson, M., Olsson, J., & Olsson, O. (2011). Mutagenesis as a Tool in Plant Genetics, Functional Genomics, and Breeding. *International Journal of Plant Genomics*, *2011*, 1-13. https://doi.org/10.1155/2011/314829

U.S. Department of Agriculture (USDA). (2024). Montana 2024 wheat varieties. U.S. Department of Agriculture, National Agricultural Statistics Service. https://www.nass.usda.gov/Statistics_by_State/Montana/index.php

U.S. Department of Agriculture (USDA). (2024). Production - Wheat. U.S. Department of Agriculture, Foreign Agricultural Service. Retrieved February 27, 2025, from https://www.fas.usda.gov/data/production/commodity/0410000

U.S. Department of Agriculture (USDA). (2024). Small grains 2024 summary. U.S. Department of Agriculture, Economics, Statistics, and Marketing Information System. https://usda.library.cornell.edu/concern/publications/5t34sj573

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

Walker, A. R., Lee, E., Bogs, J., McDavid, D. A., Thomas, M. R., & Robinson, S. P. (2007). White grapes arose through the mutation of two similar and adjacent regulatory genes. *The Plant Journal*, *49*(5), 772-785. https://doi.org/10.1111/j.1365-313X.2006.02997.x

CHAPTER TWO

THE *GRAIN NUMBER INCREASE 1* ALLELES *GNI-A1*-105Y AND -105K INCREASE GRAIN NUMBER IN SPRING WHEAT

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Abstract

Wheat *(Triticum aestivum L.)* has inflorescences made up of multiple spikelets arranged along a central rachis, with each spikelet producing between one and four grains. The *Grain Number Increase 1 (GNI-A1)* gene wheat directly influences grain number per spikelet and grain size. Three naturally occurring alleles have been described previously: *GNI-A1*-105N, 105Y, and 105K. This project's goal was to characterize the impact of these alleles within hard red spring wheat cultivars in Montana, where each of the alleles is common. The 105N allele and the 105K allele were compared through analysis of an F_5 Vida by Spring-Yellowstone recombinant inbred line (RIL) population, and with near isogenic lines (NIL) derived from the same population. The 105N allele and the 105Y allele were compared with NILs derived from an F_4 Lanning by Egan RIL population. We analyzed the impact of each of the three alleles and compared their effects on inflorescence architecture, grain size, grain yield, grain quality, and milling quality under Bozeman, MT field conditions. Data shows that either loss-of-function alleles (105Y and 105K) increased grain number per spikelet by 5 % when compared to the more functional allele (105N) across all years and environments tested. Overall grain size was not significantly reduced, however there was also not a significant increase in overall grain yield.

Introduction

Wheat *(Triticum aestivum* L.*)* is one of the most widely cultivated crops comprising 20 % of the world's food supply (Enghiad et al., 2017; Sakuma et al., 2019). Increasing wheat yield while ensuring its nutritional composition and quality characteristics remain constant or improved is one of the foremost challenges in agriculture (Erenstein et al., 2022). Different steps

in the development and maturation of a wheat plant can be manipulated via plant breeding to increase yield. Increased grain size, grain number per spike, and spike number are common metrics that breeders select for to increase yield while attempting to maintain or increase protein content (Quintero et al., 2018; Tillett et al., 2022). Manipulating grain size affects wheat quality parameters in different ways. Decreased grain size is generally associated with decreased milling yield (Ficco et al., 2020; Marshall et al., 1986). Smaller grains are also associated with an increase in grain and flour protein content. Decreased milling yield is undesirable from a miller's perspective however, increased protein content is desirable for bread wheats because it improves end product quality and adds economic value (Baasandorj et al., 2015; Hogg & Giroux, 2019). In general, it is difficult to increase total grain yield per spike through the manipulation of any one specific gene, since grain size and grain number per spike are often inversely related (Tillett et al., 2022; Xie & Sparkes, 2021). Understanding the genetic relationship between these two specific traits is important when attempting to alter wheat spike architecture and grain traits. Wheat and other cereal crops in the *Triticeae* family produce inflorescences centered around an unbranched rachis with one to three spikelets growing from each rachis node. Each spikelet is made up of multiple florets alternately spaced along a central rachilla. (Sakuma & Schnurbusch, 2020) Each spikelet may have as many as six individual florets, but only fertile florets will produce a grain. The number of fertile florets per spikelet directly impacts grain yield (Golan et al., 2019; Sakuma et al., 2019; Tillett et al., 2022). It has generally been accepted that increasing the number of potential grains per unit area through an increase in productive spikes or in potential grain number per spike during plant development prior to flowering has a positive impact on yield (Ferrante et al., 2020). After flowering, since the overall number of grains is

relatively fixed, so increasing grain size by increasing potential for increased grain fill has the biggest impact on yield (Reynolds et al., 2022; Slafer et al., 2023). While length and rate of grain fill is known to affect grain size and overall grain yield, longer grain fill periods are not associated with larger grain size, especially in environments that experience heat and drought stress during this period (Bruckner & Frohberg, 1987; Lizana et al., 2010; Xie et al., 2015). Other plant traits can indirectly affect these considerations, for example height is oftentimes associated with higher grain number but smaller grains, and larger flag leaf size being associated with larger grains (Ali et al., 2010; Khaliq et al., 2008). In general, increasing the number of grains decreases the ability of a plant to increase grain size, and vice versa. However, this convention has been contradicted by recent studies that have selectively upregulated expansins in developing seeds after flowering, showing a positive impact on grain size and a direct positive impact on grain yield with no decrease in grain number (Calderini et al., 2021). These results magnify the importance of studying genes that may help to increase potential grain number, since beneficial grain number alleles could be successfully combined with alleles of other genes that increase seed size, resulting in a net positive for grain yield.

The *GNI-A1 (Grain Number Increase-A1*) locus was first identified as a QTL for grain number per spike on chromosome arm 2AL (Guo et al., 2017). The underlying polymorphic gene was later discovered to be a transcription factor that plays a key role in the tradeoff relationship between grain size and number per spike by affecting floret fertility (Sakuma et al., 2019). *GNI-A1* is a HOX-1 class transcription factor that promotes floret abortion, belonging to a family of genes known as Homeodomain Leucine Zipper (HD-Zip) Transcription Factors, which suppress organ development (Gonzalez-Grandio et al., 2017). This gene is directly orthologous to the

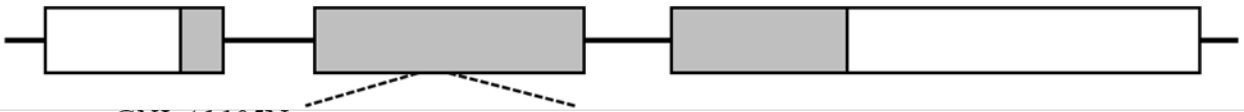
more well studied gene *Vrs1 (Six Rowed Spike-1*) in Barley (*Hordeum vulgare*), and is more distantly orthologous to *Grassy Tillers 1 (GT1)* in maize (*Zea mays*) (Bull et al., 2017; Sakuma et al., 2019; Sakuma et al., 2013)(Dong et al., 2019; Klein et al., 2022; Whipple et al., 2011). Additionally, other orthologs have been described in other monocots and dicots including rice (*Oryza sativa*), *Brachypodium* (*Brachypodium distachyon*), and *Arabidopsis* (*Arabidopsis thaliana*) (Perotti et al., 2017; Sakuma et al., 2010; Shao et al., 2018).

Across the three *GNI1* homoeologs in wheat, there is expression of *GNI-A1* and *GNI-D1* in spike tissue, but very little detectable expression of *GNI-B1*. Expression of *GNI-A1* is four times higher than that of *GNI-D1* in spike tissue. No allelic variation in *GNI-D1* among modern cultivars has been reported (Tillett et al., 2022). Three different *GNI-A1* alleles have been reported among 210 hexaploid winter wheat cultivars (Sakuma et al., 2019). The ancestral or wildtype allele is defined by the encoding of an Asparagine (N) at the 105th codon. Two different missense alleles have been reported in modern wheat, each containing a single amino acid change at this codon. *GNI-A1*-105Y encodes a Tyrosine, and *GNI-A1*-105K encodes a lysine at this position (Figure 2.1). The *GNI-A1*-105Y allele reduces floret abortion, resulting in more grains per spikelet and higher yields relative to the wildtype allele. This was shown through analysis of lines within a population where mutagenesis of the variety Kitahonami (which contains the 105Y allele) was employed to artificially recreate the 105N allele. M_4 lines were compared in full density field experiments (Sakuma et al., 2019). An association between the 105Y allele and higher grain number per spikelet was also shown through QTL analysis of a double haploid line population derived from a cross between parents Shunyo (containing the 105N allele) and Kitahonami (containing the 105Y allele) (Mizuno et al., 2021). No prior

research has been conducted to compare the 105K and the 105N allele. Ancestral, tetraploid emmer wheat (*Triticum dicoccum)* varieties were shown to have higher expression of *GNI-A1* resulting from two gene copies in the A genome, while modern tetraploid durum wheat (*Triticum durum*) cultivars were shown to have one gene copy on the A genome with half the expression of *GNI-A1, and* larger grains, but fewer grains per spikelet as a result (Golan et al., 2019). *GNI-A1* can be described in modern wheat cultivars as controlling the tradeoff between grain size and grain number per spikelet, with functional alleles resulting in larger but fewer grains per spikelet, and decreased function alleles resulting in smaller, but more grains per spikelet (Sakuma et al., 2019; Sakuma & Schnurbusch, 2020).

The aims of this study were to determine the distribution of *GNI-A1* 105N, 105Y, and 105K alleles in Montana adapted wheat varieties, to investigate the impact of the 105K allele in an RIL population, to confirm the discovered effects of the 105K allele along with the previously published effects of the 105Y allele using near isogenic lines, and to determine whether or not these alleles have an effect on grain yield and end use quality traits.

Figure 2.1. Graphical representation of polymorphism between *GNI-A1* alleles. The ancestral allele of GNI-A1 encodes an Asparagine (N) at the 105th codon, while missense alleles *GNI-A1*-105Y encodes a Tyrosine, and *GNI-A1*-105K encodes a lysine.



GNI-A1 105N VAVWFQ**N**RRARHK
GNI-A1 105Y VAVWFQ**Y**RRARHK
GNI-A1 105K VAVWFQ**K**RRARHK

Materials And Methods

Variety Screen Methods

Thirty-two varieties of hexaploid wheat and 12 varieties of tetraploid wheat commonly grown in Montana were checked for polymorphism in the *GNI-A1* gene utilizing published PCR primers and protocols (Sakuma et al., 2019). To rule out the possibility of additional *GNI-1* variation resulting from different alleles of the *GNI-D1* homoeolog, an additional screen was performed across the 32 hexaploid wheat varieties. Two additional primers and a protocol were designed to aid in sequencing of *GNI-D1*. Sequence differences between the three *GNI1* homoeologs were analyzed manually to come up with two new GNI-D1 specific primers: GNI-DexF 5'-AACTGAACTTTATCGACCG-3', and GNI-DexR 5'-TGGGAGAAACGATTTAGC-3'. The previously reported primer GNI-A1EPF was also used (Sakuma et al., 2019). A nested PCR was performed in which pre-amplification of the entire gene was followed by an internal amplification of specific regions. PCR products were then Sanger sequenced and screened for polymorphisms. PCR conditions for GNI-D1 pre-amplification was 40 cycles of 30 sec at 96° C, 30 sec at 60° C, and two min at 72° C, with an expected amplicon size of about 1600 base pairs. Each 25 µl pre-amplification reaction consisted of 1.2 µl of genomic DNA at a concentration of approximately 2 µg/µl, 13.67 µl of ultrapure nuclease-free water, 5 µl of 5x Green GoTaq® Flexi Buffer (Promega, Madison, WI), 2 ul of $MgCl_2$ at a concentration of 25 mM, 2 µl of dNTP at a concentration of 2 mM, 0.5 µl of 20 µm Primer GNI-DexF, 0.5 µl of 20 µm Primer GNI-DexR, and 0.13 µl of GoTaq® G2 Flexi DNA Polymerase (Promega, Madison, WI). PCR conditions for the GNI-D1 internal amplification was 40 cycles of 30 sec denaturation at 96° C, 30 sec of annealing at 55° C, and two min of extension at 72° C, with an expected amplicon size of about

1500 base pairs. Each 25 µl internal amplification reaction consisted of 1.2 µl of a 1:100 dilution of PCR product from the pre-amplification,, 13.67 µl of ultrapure nuclease-free water, 5 µl of 5x Green GoTaq® Flexi Buffer (Promega, Madison, WI), 2 ul of $MgCl_2$ at a concentration of 25 mM, 2 µl of dNTP at a concentration of 2 mM, 0.5 µl of 20 Mm Primer GNI-A1EPF, 0.5 µl of 20 Mm Primer GNI-DexR, and 0.13 µl of GoTaq® G2 Flexi DNA Polymerase (Promega, Madison, WI). Sanger Sequencing using both Primer GNI-A1EPF and Primer GNI-DexR as sequencing primers was performed by Azenta/GENEWIZ (Azenta US, Inc., South Plainfield, NJ, USA). Sequence was aligned and analyzed in SeqMan Pro® Version 17 (DNASTAR, Madison, WI) with variant nucleotide peak discovery threshold set to 30 %. (See Supplementary Table A1.1 for a list of all primers used in this study.)

Once it was determined that only previously reported *GNI-A1* alleles were present among the screened MT cultivars, all populations created by intercrossing varieties containing the different known alleles were genotyped by sequencing across the *GNI-A1*-105th codon position. (See figure 2.1 for a representation of amino acid polymorphism between alleles.)

Development of Plant Material

To determine if there were any realized phenotypic differences in lines containing the *GNI-A1* 105-K allele compared to the 105N allele, a recombinant inbred line (RIL) population segregating for the respective alleles was genotyped and grown in the field in Bozeman, MT in 2021 and 2022. The RIL population in this study was derived from a cross between cultivars hard red spring Vida (PI642366) and a spring habit version of the hard red winter variety Yellowstone (PI 643428). The spring habit Yellowstone (PI 643428) was created by marker assisted backcrossing of the spring habit allele of the *VRN-A1* gene into Yellowstone (Bruckner

et al., 2007; Cook et al., 2018; Lanning et al., 2006). Vida contains the 105K allele and Spring-Yellowstone contains the 105N allele. One hundred forty-six different lines were advanced by single seed decent to the F_5 generation and grown in head rows. Bulk samples of $F_{5:6}$ grain from these head rows was grown and genotyped. Seventy-six of these lines were fixed for the *GNI-A1*-105N allele, 66 were fixed for the *GNI-A1*-105K allele, and four were heterogeneous lines.

The RIL population in this study varied for many other traits, so to isolate variability to the gene of interest, Near Isogenic Lines (NIL's) from different heterozygous inbred families (HIFs) (Tuinstra et al., 1997) were generated and grown in the field in Bozeman, MT in 2022. To create NILs to directly compare the *GNI-A1*-105K and *GNI-A1*-105N alleles, two F_5 Vida/Spring-Yellowstone RILs segregating for *GNI-A1* were selected as HIFs. These families are identified as Vida/Spring-Yellowstone 34 and Vida/Spring-Yellowstone 87. From these F_5 families, several F_6 single plants were grown and genotyped to identify individual plants that were heterozygous for *GNI-A1*-105N and *GNI-A1*-105K alleles. Twenty F_7 seeds from each of the two F_6 heterozygous plants representing the two HIF families were grown. Three plants homozygous for *GNI-A1*-105N, and three plants homozygous for *GNI-A1*-105K within each of the two HIF families were randomly selected as NILs. F_8 seed from these individual F_7 plants was increased in Yuma, AZ. $F_{7:9}$ seed was used to plant experimental plots in Bozeman in 2022.

Another set of NIL lines were generated in order to confirm phenotypic differences between lines containing the *GNI-A1* 105N and 105Y alleles within populations with isolated variability. A Lanning (PI 676978) by Egan (PI 671855) F_4 RIL population was screened for *GNI-A1* alleles. Lanning and Egan are both Montana adapted hard red spring wheat lines (Blake et al., 2014; Heo et al., 2016). Lanning contains *GNI-A1*-105N, and Egan contains *GNI-A1*-105Y.

Four F_4 RIL's were found to be segregating for *GNI-A1* and were selected as HIF's. These families are identified as Lanning/Egan 44, Lanning/Egan 68, Lanning/Egan 71, and Lanning/Egan 82. NILs were derived from each of these families, as in the Vida/Spring-Yellowstone lines. Three F_6 plants homozygous for each allele were randomly selected from each family. After increase, $F_{6:8}$ seed was used to plant experimental plots in Bozeman in 2022.

This RIL population was grown in 2022 and 2023, while the two groups of NIL lines were grown in 2022 only. Vida, Spring Yellowstone, Lanning, and Egan, from which these populations were developed, are all modern, high yielding cultivars adapted to a Montana environment, so while these lines vary morphologically, any linkage effects associated with any of these cultivars would not be considered to have a negative effect on overall grain yield.

Field Experiment Methods

All experiments were grown at the Montana State University Post Agronomy Farm near Bozeman, MT. The Vida/Spring-Yellowstone RIL population was planted in a randomized complete block design with two replications. The RIL trial was planted in two separate but adjacent, rainfed and irrigated experiments in 2021 and 2022. The NIL lines from Vida/Spring-Yellowstone and Lanning/Egan were grown in a randomized complete block design with two replications in rainfed and irrigated trials. Seeds were sown to a depth of 5 cm. The RIL population plots consisted of two 3-m rows spaced 30 cm apart, and the NIL population plots consisted of four 3-m rows spaced 30 cm apart. Seeding rate for all experiments was 3.3 g of seed per meter of row.

In the 2021 growing season, the irrigated RIL trial was planted on 21 April and the rainfed trial was planted on 22 April. Before planting, 289 kg/ha of Urea (46-0-0) was applied.

Between 1 May and 31 August, the Post farm received 17.2 cm of precipitation. The highest recorded air temperature across the growing season was 37.2 °C on August 8th, and the lowest recorded air temperature was -4.4 °C on May 22nd. (NOAA, https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00241047/). On 22 and 28 June and 5 July approximately 5 cm of irrigation water was applied using hand line sprinklers. On 6 June 1.75 l/ha of Vendetta® (31.7 % 3,5-dibromo-4-Hydroxybenzonitrile, 34 % 2-Ethylhexyl ester of 2-methyl-chlorophenoxyacetic acid Wilbur-Ellis Co. Fresno, CA USA) and 0.77 l/ha of Parity™ (11.3 % Fenoxaprop-P-Ethyl, Tenkoz Inc. Alpharetta, GA USA) were applied for disease/weed control.

In the 2022 growing season, the RIL population irrigated and rainfed trials were planted on 6 May. The irrigated NIL population trial was planted on 18 May and the rainfed NIL trial was planted on 27 May. From 1 May to 31 August, the research station received 19.8 cm of precipitation. The highest recorded air temperature across the growing season was 33.8 °C on 1 August, and the lowest recorded air temperature was -3.8 °C on 9 May. (NOAA, https://www.ncdc.noaa.gov/cdoweb/datasets/GHCND/stations/GHCND:USC00241047/detail). On 21 and 26 June, and 2 July, approximately 5 cm of irrigation water was applied using hand line sprinklers. Before planting, 174 kg/ha of Urea (46-0-0) was applied. On June 3rd, 0.05 l/ha of Affinity® TankMix (40 % Thifensulfuron-methyl, 10 % Tribenuron methyl, E.I. DuPont de Nemours and Co. Wilmington, DE USA), 0.50 l/ha MCPE (68.7 % 2-methyl-4-chlorophenoxyacetic acid isocytl (2-ethylhexyl) ester Agriliance, LLC. St. Paul MN USA), and 1.17 l/ha of Discover® (6.4 % Clodinafop-propargyl, Syngenta Crop Protection LLC. Greensboro, NC USA) were applied for weed and disease control.

Although heading date, maturity date, flag leaf length, and plant height are not known to be affected by the *GNI1* gene, these traits were measured since can directly affect grain size and grain number (Ali et al., 2010; Khaliq et al., 2008; Xie et al., 2015). Heading date was recorded for all plots as the number of days after 1 January on which approximately 50 % of the primary spikes were emerged. Maturity date was recorded as the number of days after 1 January on which approximately 50 % of the peduncles in each plot turned brown. Flag leaf length from the stem to the tip of the leaf, and width at the widest part of the leaf were recorded for three random primary flag leaves in each plot and averaged. Plant height from ground level to the top of the tallest spike (excluding awns) was recorded in two different places in each plot and averaged.

Spike morphology analysis was conducted on primary spikes, and grain traits were analyzed on bulk grain samples. Spike length from the base of the first spikelet to the top of the apical spikelet (excluding awns), was recorded for three spikes in each plot and averaged. Primary spikes were collected randomly from each plot for post-harvest analysis. The selection of primary spikes was based on picking representative spikes that exhibited greater height and maturity when compared to other spikes in the plot. Five spikes were collected per plot from the NIL populations and three spikes were collected per plot from the RIL population. Total spikelet count and sterile spikelet count were recorded for each of these spikes. After all spikelets were counted, the spikes from each individual plot were hand threshed together and grains were collected in a single envelope. A total weight and total grain count were measured for the spikes from each plot. From this data, average grains per primary spike, average grain yield per primary spike, average primary spike single grain weight, average fertile spikelets per primary spike, average grains per fertile spikelet, and average grain yield per fertile spikelet were calculated.

Plots were harvested with a Wintersteiger Nurserymaster small plot combine (Wintersteiger, Salt Lake City, UT), and grain yield was recorded.

Grain Quality Analysis Methods

For all samples, whole grain protein and moisture content were measured by near-infrared transmittance using a Foss Infratec 1241 Grain Analyzer (Foss North America, Silver Springs, MD). In the RIL population, individual grain weight was calculated using a seed counter and a sample of 200 grains. For the two NIL populations, individual grain weight, grain diameter, and kernel hardness index (KHI) was measured on a Single Kernel Characterization System 4100 (Perten, Springfield, IL, USA; AACCI Method 55-31.01). For the two NIL populations, grain samples were analyzed for flour quality. After moisture content analysis on the Foss Infratec 1241 Grain Analyzer, grain was tempered to 14.5 % moisture (AACC Method 26-10.02) before milling in a Brabender Quadromat Senior grain mill (C.W. Brabender Instruments, South Hackensack, NJ) to obtain straight grade flour. Flour ash (FASH) was obtained using the AACC Approved Method 08-01.01. Percent flour yield was calculated by dividing total flour yield by the sum of the total flour, bran, and shorts/middlings x 100. Percent bran was calculated by dividing the total bran yield by the sum of the total flour, bran, and shorts/middlings x 100.

Statistical Analysis

All response variables for the Vida/Spring-Yellowstone RIL population were analyzed via mixed model analysis of variance using the lme4 package (Bates et al. 2015) in R (R Foundation for Statistical Computing. Version 4.0.5. Vienna, Austria). Each year by experiment within year combination was treated as an environment. The model included environment, block within year, *GNI-A1* allele class, lines within *GNI-A1* allele class, and their interactions with environment. All

factors were considered fixed except lines within *GNI-A1* allele class and its interaction with environment which were considered as random effects. The Vida/Spring-Yellowstone NILs and Egan/Lanning NILs were analyzed separately within each of the two backgrounds, but averaged across the HIFs within each background using a mixed linear model with the lme4 package in R. The model for each NIL population included environment, block within environment, HIF family, *GNI-A1* allele class, lines within each HIF family by *GNI-A1* allele class combination, and interaction with environment except for those with replication within environment. All factors were fixed except for lines within family by *GNI-A1* class combination, and its interaction with environment, which were each considered random effects.

Results And Discussion

Variety Screen Results

Out of 17 spring wheat varieties, four contained the *GNI-A1*-105N allele, 9 contained the *GNI-A1*-105Y allele, and four contained the *GNI-A1*-105K allele. Out of the 15 winter wheat varieties screened, 11 contained the *GNI-A1*-105N allele, four contained the *GNI-A1*-105Y allele, and none contained the *GNI-A1*-105K allele. Durum wheat (*Triticum durum*) lines were also screened. Two related durum varieties, Carpio (PI 670039) and MT Blackbeard (PI number pending), contained the *GNI-A1*-105N allele while all other screened durum varieties carried the *GNI-A1*-105Y allele (Elias et al., 2015). (See Supplementary Table A2.2 for a list of screened varieties). Sequencing of the *GNI-D1* homoeolog across all screened hexaploid varieties did not detect any polymorphism.

RIL Results

Allelic class means (105N and 105K) means combined over all environments are reported for traits in the Vida/Spring-Yellowstone RIL population (Table 2.1). In the RIL population, plant height, spike length, yield, bulk single grain weight, and primary spike single grain weight all had significant genotype by environment interactions. These interactions were often the result of one of the four environments showing no difference between genotype classes. (See Supplementary Table A2.3 for data from each separate environment).

Averaged across all environments, lines with the *GNI-A1*-105K allele had longer flag leaves, more grains per spikelet, more grains per primary spike, higher yield per primary spike, and smaller primary spike single grain weight when compared to the *GNI-A1*-105N allele. Heading date and maturity date were similar between the two allele classes.

Table 2.1. Vida/Spring-Yellowstone RIL Population data, averaged across two years and two environments in each year. a Denotes the number of lines in each genotype class. Values represent the average for each genotype ± standard error. RILs with the *GNI-A1*-105K allele had more grains per spikelet, and slightly reduced primary spike single grain weight, although this did not translate to an overall grain yield increase.

GNI-A1		**Height**	**Flag Leaf Length**	**Flag Leaf Width**	**Yield**	**Protein Content**	**Bulk SGW**
Allele	N[a]	cm	cm	cm	Kg/Ha	Percent	mg
105N	76	87.9±0.65	**17.0±0.34**	1.34±0.02	6213±86	13.9±0.06	30.4±0.3
105K	66	88.9±0.69	**17.7±0.35**	1.33±0.03	6218±89	13.8±0.07	29.9±0.3
Pr(>F)		0.26	**0.024**	0.67	0.96	0.10	0.22

GNI-A1		**Spike Length**	**Fertile Spikelets**	**Grains/ Spikelet**	**Primary Spike SGW**	**Grains/ Primary Spike**	**Yield/ Primary Spike**
Allele	N[a]	cm	No./Spike	No./Spikelet	mg	No./Spike	g
105N	76	8.88±0.1	16.9±0.1	**2.73±0.02**	**33.6±0.3**	**46.1±0.5**	**1.54±0.02**
105K	66	9.02±0.1	17.0±0.1	**2.88±0.02**	**32.7±0.3**	**48.9±0.6**	**1.59±0.02**
Pr(>F)		0.20	0.55	**<0.0001**	**0.041**	**<0.001**	**0.020**

NIL Results

Allelic class means combined over all environments are reported for the two HIF derived NIL populations (Table 2.2 and 2.3, see Supplementary Table A2.4 for data from each separate environment). The magnitude of difference between allele classes of some traits is different among the different NIL populations, indicating that genetic background has an influence on the phenotypic differentiation between alleles of this gene. The HIF family Lanning/Egan 68 showed the largest difference in grains per spikelet between allele classes (Figure 2.2).

In the NIL populations comparing the *GNI-A1*-105N and *GNI-A1*-105K alleles, the *GNI-A1*-105K allele conferred a 2.8 % increase in height ($P < 0.0001$), a 5.7 % increase in flag leaf length ($P < 0.05$), a 5.1 % increase in grains per spikelet ($P < 0.05$), and a 3.3 % decrease in primary spike single grain weight ($P < 0.1$). Heading date and maturity date were similar between genotypes within each NIL population. There was a significant genotype by environment interaction for primary spike single grain weight. This data is consistent with findings in the RIL population. The *GNI-A1*-105K allele conferred an increase in milling yield by 1.2 % ($P < 0.001$) and a decrease in bran percent by 0.8 % ($P < 0.1$). There was no difference in flour ash content between the two alleles.

In the NIL populations comparing the *GNI-A1*-105N and *GNI-A1*-105Y alleles, the *GNI-A1*-105Y allele conferred a 5.5 % increase in grains per spikelet ($P < 0.01$) and a 1.9 % decrease in primary spike single grain weight ($P = 0.1$). However, this population did not show differences in plant height or leaf length. There were no significant genotype by environment interactions for the NIL populations comparing the *GNI-A1*-105N and the *GNI-A1*-105Y alleles. The 105Y allele conferred a decrease in milling yield by 0.5 % ($P = 0.01$) and an increase in bran percent by 0.4

% ($P < 0.05$). The 105Y allele also conferred a 2 % increase in kernel hardness ($P < 0.1$). There was no difference in flour ash content between the two alleles.

Figure 2.2. Representative spikes from the family Lanning / Egan 68, the HIF family with the largest average difference in spike size between *GNI-A1* 105N and 105K allele classes. Primary spikes from different allele classes did not vary in spikelets per spike but did vary in seeds per spikelet resulting in an overall increase in seeds per primary spike in lines containing the *GNI-A1*-105K allele.

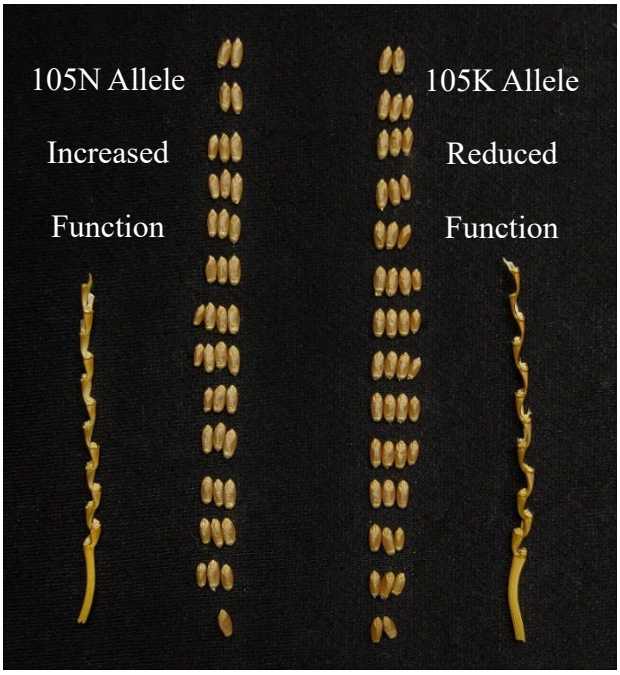


Table 2.2. Vida/Spring-Yellowstone HIF derived NIL Population data, averaged across two environments. [a] Denotes the number of lines in each genotype class. Values represent the average for each genotype ± the standard error. Height, grains per spikelet, grains per primary spike, and milling yield were all increased in Near Isogenic Lines containing the *GNI-A1*-105K allele when compared to the *GNI-A1*-105N allele.

GNI-A1		**Height**	**Flag Leaf Length**	**Flag Leaf Width**	**Yield**	**Protein Content**	**Bulk SGW**
Allele	N[a]	cm	cm	cm	Kg/Ha	Percent	mg
105N	6	**82.4±0.4**	17.6±0.3	1.30±0.02	4465±127	16.6±0.09	30.0±0.5
105K	6	**84.7±0.3**	18.6±0.3	1.28±0.02	4543±124	16.5±0.10	29.7±0.5
Pr(>F)		**<0.0001**	0.039	0.59	0.60	0.42	0.69

GNI-A1		**Spike Length**	**Fertile Spikelets**	**Grains/ Spikelet**	**Primary Spike SGW**	**Grains/ Primary Spike**	**Yield/ Primary Spike**
Allele	N[a]	cm	No./Spike	No./Spikelet	mg	No.	g
105N	6	8.88±0.1	13.5±0.1	**2.53±0.03**	30.5±0.3	**34.4±0.6**	1.05± 0.02
105K	6	9.02±0.1	13.9±0.1	**2.66±0.03**	29.9±0.3	**36.9±0.6**	1.10±0.02
Pr(>F)		0.20	0.089	**0.011**	0.062	**0.011**	0.13

GNI-A1		**Milling Yield**	**Flour Protein**	**Flour Ash**	**Bran**	**Kernel Diameter**	**Kernel Hardness**
Allele	N[a]	Percent	Percent	Percent	Percent	mm	KHI
105N	6	68.1±0.3	15.2±0.08	0.50±0.004	27.7±0.27	2.65±0.03	79.0±1.2
105K	6	69.3±0.2	15.0±0.08	0.50±0.004	26.9±0.27	2.62±0.03	77.4±1.2
Pr(>F)		<0.001	0.14	0.52	0.07	0.47	0.36

Table 2.3. Lanning/Egan HIF derived NIL Population data, averaged across 2 environments. [a]Denotes the number of lines in each genotype class. Values represent the average for each genotype ± the standard error. Grains per spikelet and grains per primary spike were increased, and milling yield was slightly reduced in Near Isogenic Lines containing the *GNI-A1*-105K allele when compared to the *GNI-A1*-105N allele

GNI-A1 Allele	N[a]	**Height** cm	**Flag Leaf Length** cm	**Flag Leaf Width** cm	**Yield** Kg/Ha	**Protein Content** Percent	**Bulk SGW** mg
105N	10	82.0±0.3	18.3±0.3	1.35±0.02	4794±77	16.9±0.09	30.6± 0.3
105Y	10	82.7±0.3	18.2±0.3	1.37±0.02	4864±81	16.7±0.09	30.2±0.3
Pr(>F)		0.20	0.83	0.38034	0.60	0.18	0.33

GNI-A1 Genotype	N[a]	**Spike Length** cm	**Fertile Spikelets** No./spike	**Grains/ Spikelet** No./Spikelet	**Primary Spike SGW** mg	**Grains/ Primary Spike** No./Spike	**Yield/ Primary Spike** g
105N	10	8.88±0.1	14.5±0.1	**2.90±0.03**	31.4±0.3	**42.1±0.6**	1.32± 0.02
105Y	10	9.02±0.1	14.6±0.1	**3.06±0.04**	30.8±0.3	**44.5±0.7**	1.36±0.02
Pr(>F)		0.20	0.64	**0.0094**	0.10	**0.025**	0.14

GNI-A1 Allele	N[a]	**Milling Yield** Percent	**Flour Protein** Percent	**Flour Ash** Percent	**Bran** Percent	**Kernel Diameter** mm	**Kernel Hardness** KHI
105N	10	**69.79±0.08**	15.4±0.1	0.47±0.004	26.5±0.1	2.67±0.1	80.7±0.6
105Y	10	**69.31±0.09**	15.4±0.1	0.48±0.004	26.9±0.1	2.68±0.1	82.3±0.6
Pr(>F)		**0.010**	0.44	0.20	0.018	0.91	0.083

Discussion

Previous research using EMS mutagenesis populations has shown that *GNI-A1*-105Y confers an increase in grains per spikelet, but there has not been conclusive research on whether similar increases are conferred by the *GNI-A1*-105K allele (Golan et al., 2019; Mizuno et al., 2021; Sakuma et al., 2019). This research confirms published grain number increase effects of the 105Y allele using NIL populations, although the differences observed were relatively small, and no significant yield increase was observed. *GNI-A1*-105K lines had more grains per spikelet when compared with *GNI-A1*-105N across all environments and populations. This indicates that

the *GNI-A1*-105K allele is most likely a decreased *GNI-A1* functional allele that results in lower instances of floret abortion resulting in more grains per spikelet, similar to the *GNI-A1*-105Y allele, although analysis of *GNI-A1* transcriptional activity between alleles would need to be done to confirm the genetic underpinnings of these findings.

There was an observed difference in the single grain weight of primary wheat spikes between lines with different *GNI-A1* alleles. However, there was a much smaller difference observed in bulk sample single grain weight, representing grains from all harvested spikes. The bulk grain size results are consistent with (Sakuma et al., 2019) which showed that *GNI-A1* RNAi induced knockouts increased grain number per spike but did not show a decrease in grain size. Inconsistencies between primary head and bulk sample grain size indicate a limitation to this study, as the differences caused by *GNI-A1* variation may not be as strongly realized in secondary spikes as opposed to primary spikes (Table 2.2 and Table 2.3). A more complete analysis of secondary spikes may be needed to determine the impacts of *GNI-A1* more generally. No significant yield differences were seen in these experiments, although the natural mutant alleles may be associated with a slight upward trend in yield. The lack of significant overall yield increase, despite an increase in grain number per spike without a large reduction in grain size, could be because of a smaller change in grains per spikelet in secondary spikes, or could be because of a slight decrease in productive tiller number which was not measured. In addition, the variety screen showed that these three naturally occurring alleles are all relatively evenly distributed across different spring wheat varieties in Montana, which indicates that this locus has not been subjected to selection pressure. Thus, it would make sense that none of these alleles would confer a significant yield advantage in the studied environment.

In general, single grain weight is negatively correlated with protein content (Joppa et al., 1997; Tabbita et al., 2017). However, this correlation was not observed between allele classes in this study. Although a downward trend in single grain weight was detected when comparing the *GNI-A1*-105N allele to either the 105Y or 105K allele, an increase in protein content was not observed. This suggests that manipulating grain number through the *GNI-A1* gene may not affect protein content. Milling results varied between different NIL populations and are most likely due to different linkages and between populations containing the two alleles and do not seem to correlate with changes in grain size. This could be a result of variability in milling and grain quality between the parent lines from which these populations are derived.

Small differences in overall plant architecture (height and leaf length) were also detected between genotypes in this study. Lines with the 105K allele have longer flag leaves by up to 1 cm when compared against lines with the 105N allele (Table 2.1, Table 2.3). Longer flag leaves in lines with the 105K allele is somewhat consistent with the finding that barley lines with lower functioning *VRS-1* alleles have more leaf veins and larger flag leaves (Thirulogachandar et al., 2017). However, the observed architectural differences could be an artifact of genetic linkage.

No detected polymorphism in the *GNI-D1* homoeolog is consistent with other screens, and QTL studies have not detected a phenotypic effect originating from the *GNI-D1* locus (Li et al., 2023; Lin et al., 2021). However, *GNI-D1* is expressed at around ¼ the levels of *GNI-A1* in developing spikes, indicating that expression of the *GNI-D1* homoeolog on the D genome of spring wheat could also resulting in enough remaining GNI protein function to mask any large differences. If the natural mutant alleles of *GNI-A1* do result in decreased function and not a total loss of function, full knockout of function alleles of *GNI-A1* and potentially *GNI-D1* resulting

from mutation breeding or suppressing function of the gene using RNA Interference (RNAi) may be necessary to realize the maximum grain number advantages as a direct result of the manipulation of this gene.

This study, along with another QTL study by Cao et al., shows that the *GNI-A1* locus is associated with changes in grains per spike, but does not alter yield in elite backgrounds (Cao et al., 2020). *GNI-A1*, along with other genes that increase yield potential by increasing grain number per spike, are most likely subject to epistatic interactions that cancel out yield benefits, especially in environments that commonly experience terminal drought conditions during grain fill when this yield benefit would be potentially realized (Reynolds et al., 2022; Serrago et al., 2013; Slafer et al., 2023; Snowdon et al., 2021). However, combining alleles of *GNI-A1* that increase grain number with alleles of other genes that positively impact grain size may be the next step in increasing overall grain yields (Calderini et al., 2021; Tillett et al., 2022).

Conclusion

In spring wheat, both the 105K and the 105Y alleles of *GNI-A1* confer more grains per wheat spikelet versus the 105N ancestral allele. This increase comes with a slight decrease in single grain weight, and no significant increase in overall grain yield. These alleles do not have a consistent effect on grain protein and milling traits, and thus can be selected for without decreasing grain quality.

Appendix Material

Supplemental material can be found in Appendix A

Table A2.1, Primer list: this table contains sequences and descriptions of all oligonucleotides used in PCR amplification and sequencing.

Table A2.2, *GNI-A1* Variety Screen: This table contains a list of all varieties screened for *GNI-A1* polymorphism along with supplemental information for each variety.

Table A2.3, *GNI-A1* RIL Data: This table contains least squared means split into different environments and years for all parameters measured in the RIL population.

A2.4, *GNI-A1* NIL Data: This table contains least squared means split into different environments for all parameters measured in the NIL populations.

References

Ali, M. A., Hussain, M., Khan, M. I., Ali, Z., Zulkiffal, M., Anwar, J., . . . Zeeshan, M. (2010). Source-Sink Relationship between Photosynthetic Organs and Grain Yield Attributes during Grain Filling Stage in Spring Wheat (Triticum aestivum). *International Journal of Agriculture and Biology*, *12*(4), 509-515.

Baasandorj, T., Ohm, J. B., Manthey, F., & Simsek, S. (2015). Effect of Kernel Size and Mill Type on Protein, Milling Yield, and Baking Quality of Hard Red Spring Wheat. *Cereal Chemistry*, *92*(1), 81-87. https://doi.org/10.1094/Cchem-12-13-0259-R

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

Blake, N. K., Stougaard, R. N., Bohannon, B., Weaver, D. K., Heo, H. Y., Lamb, P. F., . . . Talbert, L. E. (2014). Registration of 'Egan' Wheat with Resistance to Orange Wheat Blossom Midge. *Journal of Plant Registrations*, *8*(3), 298-302. https://doi.org/10.3198/jpr2014.04.0022crc

Bruckner, P. L., Berg, J. E., Riveland, N., Eckhoff, J. L., Wichman, D. M., Kephart, K. D., . . . Chen, X. (2007). Registration of 'Yellowstone' Wheat. *Journal of Plant Registrations*, *1*(1), 18-19. https://doi.org/10.3198/jpr2006.12.0788crc

Bruckner, P. L., & Frohberg, R. C. (1987). Rate and Duration of Grain Fill in Spring Wheat. *Crop Science*, *27*(3), 451-455. https://doi.org/DOI 10.2135/cropsci1987.0011183X002700030005x

Bull, H., Casao, M. C., Zwirek, M., Flavell, A. J., Thomas, W. T. B., Guo, W. B., . . . Waugh, R. (2017). Barley SIX-ROWED SPIKE3 encodes a putative Jumonji C-type H3K9me2/me3 demethylase that represses lateral spikelet fertility. *Nature Communications*, *8*. https://doi.org/10.1038/s41467-017-00940-7

Calderini, D. F., Castillo, F. M., Arenas-M, A., Molero, G., Reynolds, M. P., Craze, M., . . . McQueen-Mason, S. J. (2021). Overcoming the trade-off between grain weight and number in wheat by the ectopic expression of expansin in developing seeds leads to increased yield potential. *New Phytologist*, *230*(2), 629-640. https://doi.org/10.1111/nph.17048

Cao, S., Xu, D., Hanif, M., Xia, X., & He, Z. (2020). Genetic architecture underpinning yield component traits in wheat. *Theoretical and Applied Genetics*, *133*(6), 1811-1823. https://doi.org/10.1007/s00122-020-03562-8

Cook, J. P., Heo, H. Y., Varella, A. C., Lanning, S. P., Blake, N. K., Sherman, J. D., . . . Talbert, L. E. (2018). Evaluation of a QTL Mapping Population Composed of Hard Red Spring and Winter Wheat Alleles Using Various Marker Platforms. *Crop Science*, *58*(2), 701-712. https://doi.org/10.2135/cropsci2017.08.0488

Dong, Z. B., Xiao, Y. G., Govindarajulu, R., Feil, R., Siddoway, M. L., Nielsen, T., . . . Chuck, G. (2019). The regulatory landscape of a core maize domestication module controlling bud dormancy and growth repression. *Nature Communications*, *10*. https://doi.org/10.1038/s41467-019-11774-w

Elias, E. M., Manthey, F. A., & AbuHammad, W. A. (2015). Registration of 'Carpio' Durum Wheat. *Journal of Plant Registrations*, *9*(1), 78-82. https://doi.org/10.3198/jpr2014.05.0030crc

Enghiad, A., Ufer, D., Countryman, A. M., & Thilmany, D. D. (2017). An Overview of Global Wheat Market Fundamentals in an Era of Climate Concerns. *International Journal of Agronomy*, *2017*. https://doi.org/10.1155/2017/3931897

Erenstein, O., Poole, N., & Donovan, J. (2022). Role of staple cereals in human nutrition: Separating the wheat from the chaff in the infodemics age. *Trends in Food Science & Technology*, *119*, 508-513. https://doi.org/10.1016/j.tifs.2021.11.033

Ferrante, A., Savin, R., & Slafer, G. A. (2020). Floret development and spike fertility in wheat: Differences between cultivars of contrasting yield potential and their sensitivity to photoperiod and soil N. *Field Crops Research*, *256*. https://doi.org/10.1016/j.fcr.2020.107908

Ficco, D. B. M., Beleggia, R., Pecorella, I., Giovanniello, V., Frenda, A. S., & Vita, P. D. (2020). Relationship between Seed Morphological Traits and Ash and Mineral Distribution along the Kernel Using Debranning in Durum Wheats from Different Geographic Sites. *Foods*, *9*(11), 1523. https://doi.org/10.3390/foods9111523

Golan, G., Ayalon, I., Perry, A., Zimran, G., Ade-Ajayi, T., Mosquna, A., . . . Peleg, Z. (2019). GNI-A1 mediates trade-off between grain number and grain weight in tetraploid wheat. *Theoretical and Applied Genetics*, *132*(8), 2353-2365. https://doi.org/10.1007/s00122-019-03358-5

Gonzalez-Grandio, E., Pajoro, A., Franco-Zorrilla, J. M., Tarancon, C., Immink, R. G. H., & Cubas, P. (2017). Abscisic acid signaling is controlled by a BRANCHED1/HD-ZIP I cascade in Arabidopsis axillary buds. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(2), E245-E254. https://doi.org/10.1073/pnas.1613199114

Guo, Z., Chen, D., Alqudah, A. M., Röder, M. S., Ganal, M. W., & Schnurbusch, T. (2017). Genome-wide association analyses of 54 traits identified multiple loci for the determination of floret fertility in wheat. *New Phytologist*, *214*(1), 257-270. https://doi.org/10.1111/nph.14342

Heo, H. Y., Lanning, S. P., Lamb, P. F., Nash, D., Wichman, D. M., Kephart, K. D., . . . Talbert, L. E. (2016). Registration of 'Lanning' Hard Red Spring Wheat. *Journal of Plant Registrations*, *10*(3), 287-290. https://doi.org/10.3198/jpr2016.03.0016crc

Hogg, A. C., & Giroux, M. J. (2019). Milling and baking quality of hexaploid spring wheat starch synthase IIa (ssIIa) mutants with elevated amylose content. *Cereal Chemistry*, *96*(3), 532-544. https://doi.org/10.1002/cche.10153

Joppa, L. R., Du, C. H., Hart, G. E., & Hareland, G. A. (1997). Mapping gene(s) for grain protein in tetraploid wheat (Triticum turgidum L.) using a population of recombinant inbred chromosome lines. *Crop Science*, *37*(5), 1586-1589. https://doi.org/10.2135/cropsci1997.0011183X003700050030x

Khaliq, I., Irshad, A., & Ahsan, M. (2008). Awns and flag leaf contribution towards grain yield in spring wheat (Triticum aestivum L.). *Cereal Research Communications*, *36*(1), 65-76. https://doi.org/10.1556/Crc.36.2008.1.7

Klein, H., Gallagher, J., Demesa-Arevalo, E., Abraham-Juarez, M. J., Heeney, M., Feil, R., . . . Bartlett, M. (2022). Recruitment of an ancient branching program to suppress carpel development in maize flowers. *Proc Natl Acad Sci U S A*, *119*(2). https://doi.org/10.1073/pnas.2115871119

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

Li, L. L., Bian, Y. J., Dong, Y., Song, J., Liu, D., Zeng, J. Q., . . . Cao, S. H. (2023). Identification and validation of stable quantitative trait loci for yield component traits in wheat. *Crop Journal*, *11*(2), 558-563. https://doi.org/10.1016/j.cj.2022.09.012

Lin, Y., Jiang, X. J., Hu, H. Y., Zhou, K. Y., Wang, Q., Yu, S. F., . . . Liu, Y. X. (2021). QTL mapping for grain number per spikelet in wheat using a high-density genetic map. *Crop Journal*, *9*(5), 1108-1114. https://doi.org/10.1016/j.cj.2020.12.006

Lizana, X. C., Riegel, R., Gomez, L. D., Herrera, J., Isla, A., McQueen-Mason, S. J., & Calderini, D. F. (2010). Expansins expression is associated with grain size dynamics in wheat (Triticum aestivum L.). *Journal of Experimental Botany*, *61*(4), 1147-1157. https://doi.org/10.1093/jxb/erp380

Marshall, D. R., Mares, D. J., Moss, H. J., & Ellison, F. W. (1986). Effects of Grain Shape and Size on Milling Yields in Wheat .2. Experimental Studies. *Australian Journal of Agricultural Research*, *37*(4), 331-342. https://doi.org/10.1071/AR9860331

Mizuno, N., Ishikawa, G., Kojima, H., Tougou, M., Kiribuchi-Otobe, C., Fujita, M., & Nakamura, K. (2021). Genetic mechanisms determining grain number distribution along the spike and their effect on yield components in wheat. *Molecular Breeding*, *41*(10). https://doi.org/10.1007/s11032-021-01255-8

Perotti, M. F., Ribone, P. A., & Chan, R. L. (2017). Plant transcription factors from the homeodomain-leucine zipper family I. Role in development and stress responses. *IUBMB Life*, *69*(5), 280-289. https://doi.org/10.1002/iub.1619

Quintero, A., Molero, G., Reynolds, M. P., & Calderini, D. F. (2018). Trade-off between grain weight and grain number in wheat depends on GxE interaction: A case study of an elite CIMMYT panel (CIMCOG). *European Journal of Agronomy*, *92*, 17-29. https://doi.org/10.1016/j.eja.2017.09.007

Reynolds, M. P., Slafer, G. A., Foulkes, J. M., Griffiths, S., Murchie, E. H., Carmo-Silva, E., . . . Flavell, R. B. (2022). A wiring diagram to integrate physiological traits of wheat yield potential (vol 3, pg 318, 2022). *Nature Food*, *3*(8), 665-665. https://doi.org/10.1038/s43016-022-00574-z

Sakuma, S., Golan, G., Guo, Z. F., Ogawa, T., Tagiri, A., Sugimoto, K., . . . Komatsuda, T. (2019). Unleashing floret fertility in wheat through the mutation of a homeobox gene. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(11), 5182-5187. https://doi.org/10.1073/pnas.1815465116

Sakuma, S., Pourkheirandish, M., Hensel, G., Kumlehn, J., Stein, N., Tagiri, A., . . . Komatsuda, T. (2013). Divergence of expression pattern contributed to neofunctionalization of duplicated HD-Zip I transcription factor in barley. *New Phytologist*, *197*(3), 939-948. https://doi.org/10.1111/nph.12068

Sakuma, S., Pourkheirandish, M., Matsumoto, T., Koba, T., & Komatsuda, T. (2010). Duplication of a well-conserved homeodomain-leucine zipper transcription factor gene in barley generates a copy with more specific functions. *Functional & Integrative Genomics*, *10*(1), 123-133. https://doi.org/10.1007/s10142-009-0134-y

Sakuma, S., & Schnurbusch, T. (2020). Of floral fortune: tinkering with the grain yield potential of cereal crops. *New Phytologist*, *225*(5), 1873-1882. https://doi.org/10.1111/nph.16189

Serrago, R. A., Alzueta, I., Savin, R., & Slafer, G. A. (2013). Understanding grain yield responses to source-sink ratios during grain filling in wheat and barley under contrasting environments. *Field Crops Research*, *150*, 42-51.

Shao, J., Haider, I., Xiong, L., Zhu, X., Hussain, R. M. F., Övernäs, E., . . . Ouwerkerk, P. B. F. (2018). Functional analysis of the HD-Zip transcription factor genes Oshox12 and Oshox14 in rice. *PLOS ONE*, *13*(7), e0199248. https://doi.org/10.1371/journal.pone.0199248

Slafer, G. A., Foulkes, M. J., Reynolds, M. P., Murchie, E. H., Carmo-Silva, E., Flavell, R., . . . Griffiths, S. (2023). A 'wiring diagram' for sink strength traits impacting wheat yield potential. *Journal of Experimental Botany*, *74*(1), 40-71. https://doi.org/10.1093/jxb/erac410

Snowdon, R. J., Wittkop, B., Chen, T.-W., & Stahl, A. (2021). Crop adaptation to climate change as a consequence of long-term breeding. *Theoretical and Applied Genetics*, *134*(6), 1613-1623. https://doi.org/10.1007/s00122-020-03729-3

Tabbita, F., Pearce, S., & Barneix, A. J. (2017). Breeding for increased grain protein and micronutrient content in wheat: Ten years of the GPC-B1 gene. *Journal of Cereal Science*, *73*, 183-191. https://doi.org/10.1016/j.jcs.2017.01.003

Thirulogachandar, V., Alqudah, A. M., Koppolu, R., Rutten, T., Graner, A., Hensel, G., . . . Kuhlmann, M. (2017). Leaf primordium size specifies leaf width and vein number among row-type classes in barley. *The Plant Journal*, *91*(4), 601-612. https://doi.org/10.1111/tpj.13590

Tillett, B. J., Hale, C. O., Martin, J. M., & Giroux, M. J. (2022). Genes Impacting Grain Weight and Number in Wheat (Triticum aestivum L. ssp. aestivum). *Plants-Basel*, *11*(13). https://doi.org/10.3390/plants11131772

Tuinstra, M. R., Ejeta, G., & Goldsbrough, P. B. (1997). Heterogeneous inbred family (HIF) analysis: a method for developing near-isogenic lines that differ at quantitative trait loci. *Theoretical and Applied Genetics*, *95*(5-6), 1005-1011. https://doi.org/10.1007/s001220050654

Whipple, C. J., Kebrom, T. H., Weber, A. L., Yang, F., Hall, D., Meeley, R., . . . Jackson, D. P. (2011). grassy tillers1 promotes apical dominance in maize and responds to shade signals

in the grasses. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(33), E506-E512.

Xie, Q., Mayes, S., & Sparkes, D. L. (2015). Carpel size, grain filling, and morphology determine individual grain weight in wheat. *Journal of Experimental Botany*, *66*(21), 6715-6730. https://doi.org/10.1093/jxb/erv378

Xie, Q., & Sparkes, D. L. (2021). Dissecting the trade-off of grain number and size in wheat. *Planta*, *254*(1). https://doi.org/10.1007/s00425-021-03658-5

CHAPTER THREE

N-TERMINAL *RHT-A1* MUTATIONS MODIFY SPRING WHEAT PLANT HEIGHT

Contribution of Authors and Co-Authors

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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 seed size and protein content. Other alleles of *Rht-1* along with other height reducing genes have been studied to provide breeders with alternative ways to modify plant height. However, no height reducing alleles of *Rht-A1* have been characterized in hexaploid wheat. To find *Rht-A1* alleles that could be used to fine tune plant height and other phenotypic traits pleiotropically affected by *Rht-1*, an ethyl methanesulfonate (EMS) mutagenized 'Alpowa' spring wheat population was screened for *Rht-A1* N-terminal mutations. Nine mutations were identified and characterized through yeast two-hybrid (Y2H) experiments to measure the impact each mutation had upon interaction with 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 spaced-plant trials. Five of the *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, with 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 location to the function of *Rht-1*.

Introduction

Since the 1960's, the *Rht-B1b* and *Rht-D1b* alleles of the *Reduced Height-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 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, 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 seed size, 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).

Rht-1 is a DELLA protein encoding gene that 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 (Stephen Pearce et al., 2011; Ugrin et al., 2023). There are two main regions of *Rht-1*, each containing multiple conserved motifs. The GRAS domain or C terminal region of *Rht-1* contains the LHR1, VHIID, LHRII, PYFRE, and SAW motifs which are responsible for interacting with and promoting the expression of downstream plant growth restricting genes (Zentella et al., 2007). The DELLA domain or N terminal region contains the DELLA, LExLE, and TVHYNP motifs and is responsible for binding with GID1 (Figure 3.1) (Murase et al., 2008; Ueguchi-Tanaka et al., 2005). When GA3 is present, it forms a complex with GID1 and RHT-1, resulting in degradation of the RHT-1 DELLA proteins. Degradation of RHT-1 proteins effectively down-regulates growth restricting genes, increasing stem elongation.

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 (Chandler & Harding, 2013; Li et al., 2013). Missense mutations in the N terminal region of *Rht-1* cab 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. One characterized mutation known as *Rht-B1c* or the 'Tom Thumb' mutation includes an insertion in

the N terminal region 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, resulting in an extreme dwarf phenotype (Casebow et al., 2016; S. Pearce et al., 2011).

The *Rht-B1b* and *Rht-D1b* semidwarfing alleles have nonsense mutations that create stop codons in the LExLE motif of *Rht-1*. However, partial translational reinitiation at a downstream AUG codon occurs constitutively in stem tissues, creating a 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). These alleles function similarly to the *Rht-B1c* allele but show lower expression and result in a less extreme height reduction. 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 (Brown et al., 2022; Mo et al., 2018).

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). Reducing plant height while retaining drought tolerance, seedling emergence, and protein content are the main goals of these studies (Mathews et al., 2006; Schillinger et al., 1998). Natural and mutagenesis derived alternative alleles of *Rht-1* other than *Rht-B1b* and *Rht-D1b* have been characterized in all three *Rht-1* homoeologs, although no height reducing mutations resulting from N terminal mutations have been characterized in *Rht-A1* on the A genome (Flintham et al., 1997; Li et al., 2013; Mo et al., 2018).

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 traditional *Rht-1* semi dwarfing alleles on the B and D genomes, these alternative reduced height alleles can be combined with *Rht-B1b* and *Rht-D1b* or any other alternative semidwarfing *Rht* alleles to provide a greater range of height phenotypes, enabling plant breeders to fine tune plant height. These alleles provide new ways to impart reduced plant height without reducing seed size or protein content.

Materials and Methods

Allele Creation

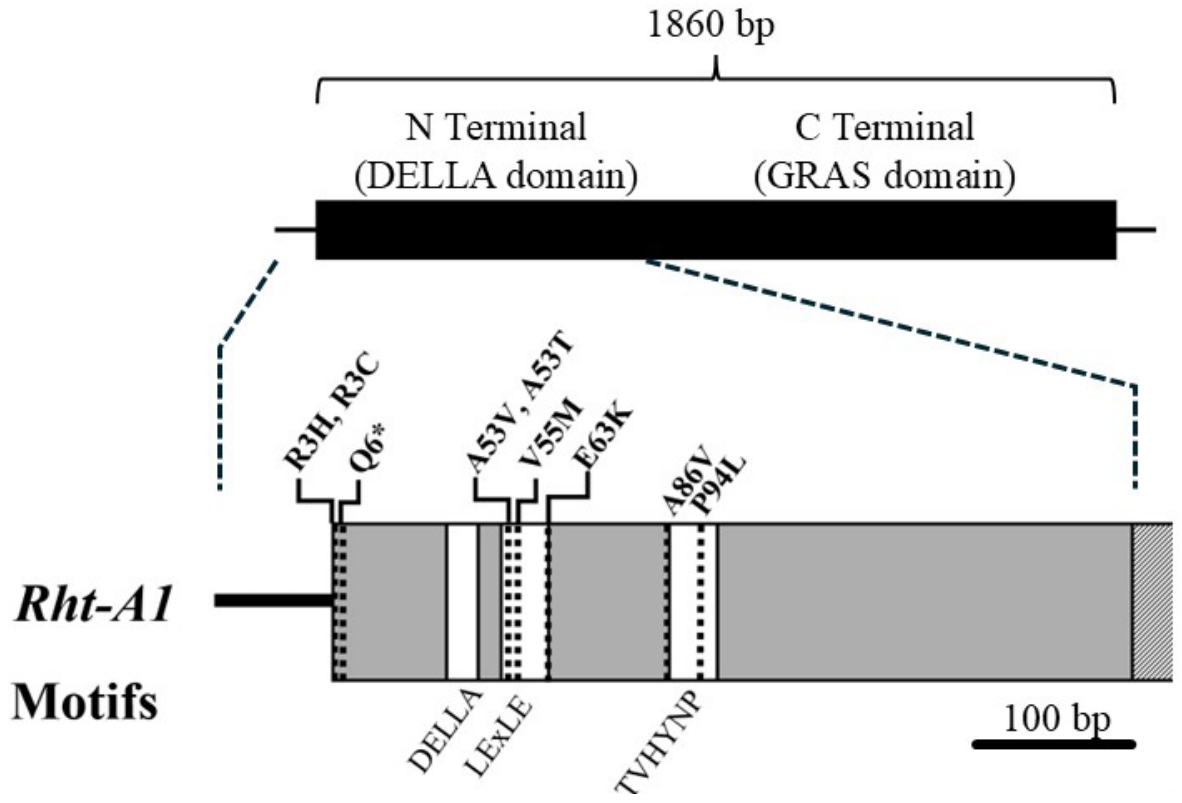
All novel alleles in this study originate from a mutagenized population created in the spring wheat cultivar 'Alpowa' (PI 566596) using ethyl methanesulfonate (EMS) as described in Feiz et al. (2009). Alpowa contains the semidwarfing allele *Rht-D1b* on the D genome, and a wildtype *Rht-B1a* allele on 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 PCR protocol described in Li et al. (2013), and PCR products were sequenced by sanger sequencing (GENEWIZ, Inc., Cambridge, MA). All induced mutations 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 3.1 and Figure 3.1).

Table 3.1. Summary of tested *Rht-A1* mutations including location, predicted effect on protein function, and summary of yeast two-hybrid results. 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

			Interaction with GID1	
Mutant Allele	**PROVEAN Score[a]**	**Predicted Effect**	**With GA3**	**Without GA3**
Rht-A1-R3C	-4.9	Deleterious	no	intermediate
Rht-A1-R3H	-2.77	Deleterious	yes	yes
Rht-A1-Q6*	NA	Deleterious	no	no
Rht-A1-A53T	-2.73	Deleterious	yes	yes
Rht-A1-A53V	-2.74	Deleterious	yes	yes
Rht-A1-V55M	-2.32	Neutral	no	yes
Rht-A1-E63K	-3.28	Deleterious	no	yes
Rht-A1-A86V	-2.31	Neutral	no	yes
Rht-A1-P94L	-8.81	Deleterious	no	yes

Figure 3.1. Visual representation of the N terminal 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.



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 were used as negative controls. 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, Mountain View, CA, USA) and the Matchmaker Gold Yeast

Two-Hybrid System (Takara Bio Inc., Mountain View, CA, USA). 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.

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. Hand line sprinklers were used to apply five centimeters of irrigation water on 21 June, 26 June, and 2 July. From 1 May 2022 to 31 August 2022, this location received 19.8 cm of precipitation (NCEI, 2025).

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, randomized complete block design with each background and tested mutation combination as the main plot, and individual NIL as the

sub plot. 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. This trial was planted on 4 May 2023, and harvested on 22 September 2023. Plots were harvested with a single row binder and threshed using a Vogel thresher. This experiment did not receive any supplemental irrigation. Between 1 May 2023 and 1 August 2023, this location received 22.0 cm of precipitation (NCEI, 2025).

In 2024, two NIL were randomly selected from each group representing every background, *Rht-B1* allele, and tested mutation (wildtype or mutant). NIL from Duclair and Vida backgrounds were 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 primary sub plot, and individual NIL as the secondary sub plot. 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. The rainfed trial was planted on 16 April 2024, and the irrigated experiment was planted 24 April 2024. Plots were harvested using a small plot combine harvester. The irrigated trial utilized hand line irrigation equipment applying approximately five centimeters of supplemental water around flowering. Between 1 April and 1 August 2024, this location received 20.3 cm of precipitation (NCEI, 2025).

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 in each plot were chosen and height was measured from the soil surface to the tip of the tallest representative 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 after 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, Silver Springs, MD).

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 NIL 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. Data from each of the 10 different populations representing each height/variety background grown in the full density trials was analyzed separately. Statistical analysis was performed on the mean values of each genotype group within each technical replicate using a mixed linear model for a randomized split plot design considering *Rht-A1* genotype, *Rht-B1* genotype, the environment, the interaction between *Rht-A1* genotype and *Rht-B1* genotype, and the interaction between *Rht-A1* genotype and the environment as fixed effects. The interaction between technical replicate and environment was considered as a random

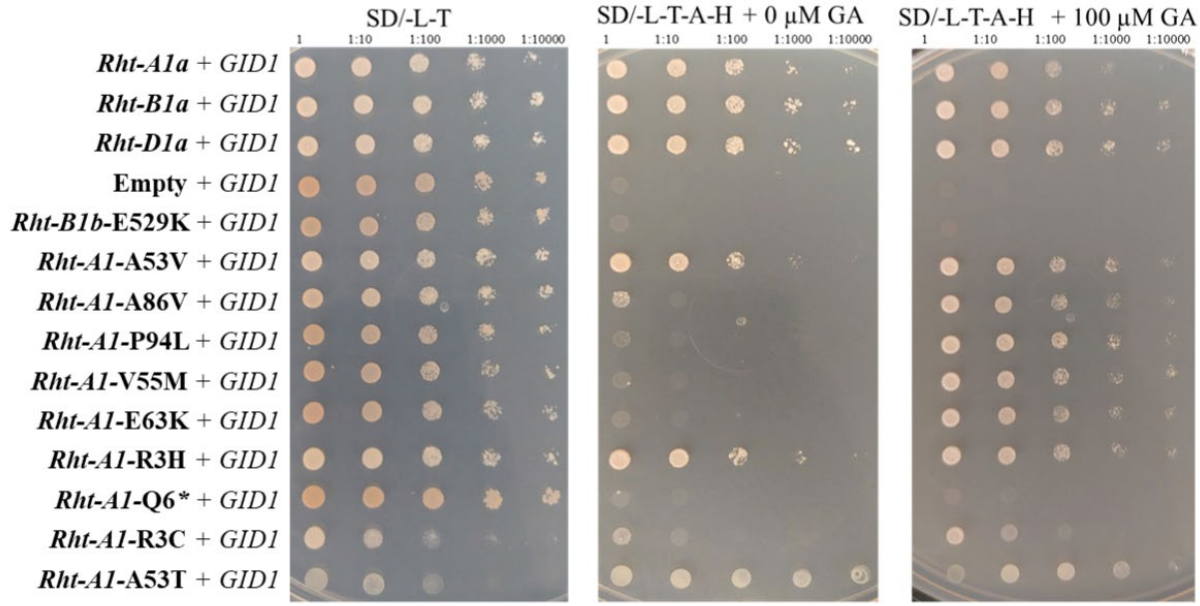
effect. The 2023 rainfed, 2024 irrigated, and 2024 rainfed experiments were considered as three separate environments. Analysis was performed using the lme4 package (Bates et al. 2015) in R (R Foundation for Statistical Computing. Version 4.0.5. Vienna, Austria).

Results

Yeast Two-hybrid 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 nine 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 3.2).

Figure 3.2. 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.



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) and the *Rht-A1*-E63K allele decreased height in the tall Duclair background by 4.2 % compared to the wild-type (p = 0.01). *Rht-A1*-A53T showed increased seed size in all four comparisons, with a significant increase of 10.2 %

in the semidwarf Duclair background ($p = 0.02$). These results contributed to these lines being chosen for inclusion in the full density trials.

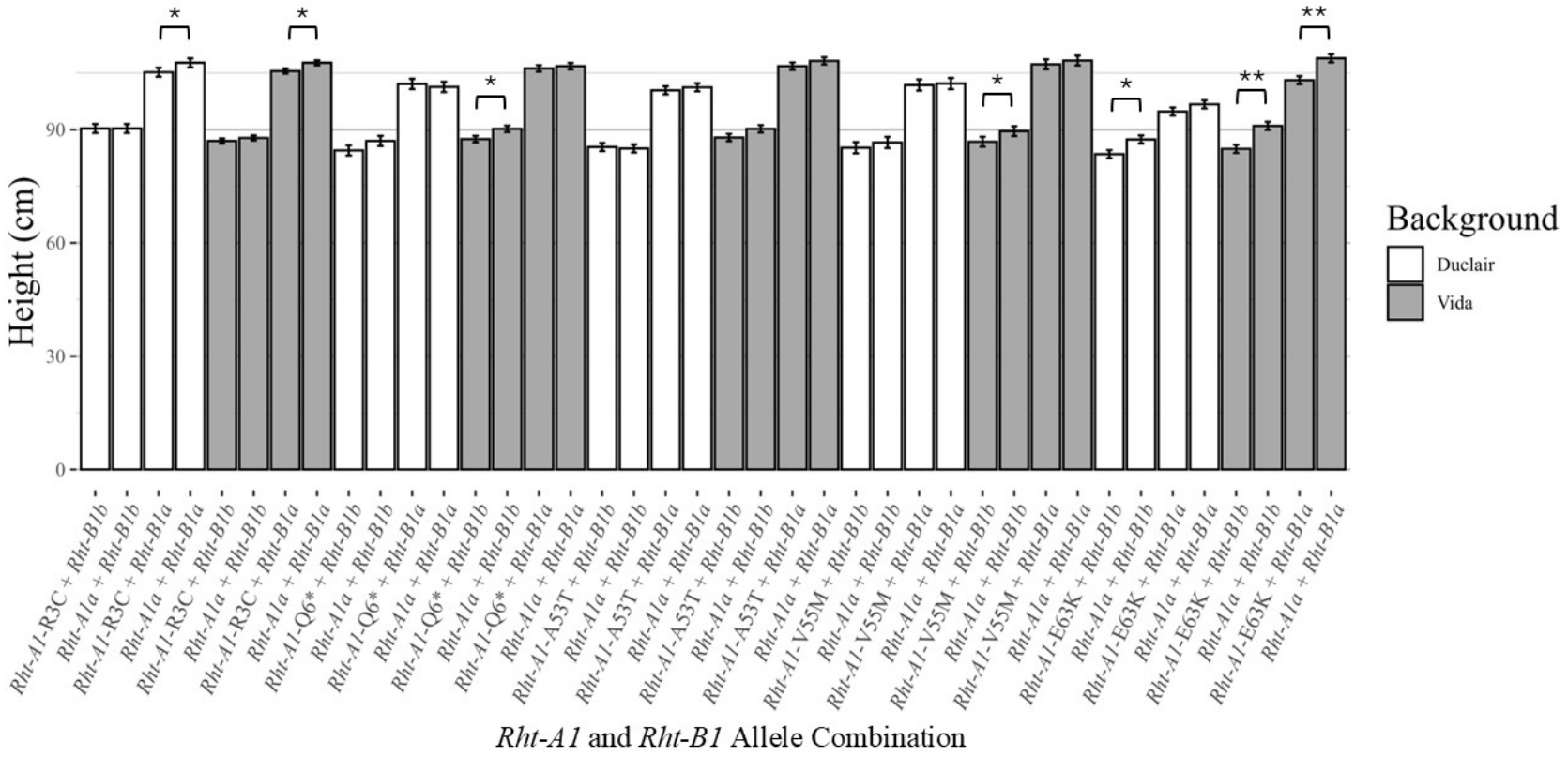
Standard Planting Density Field Study

Each tested *Rht-A1* mutation included in the full density trials contributed to a small decrease in height in both semidwarf and tall backgrounds (Figure 3.3). 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 towards an increase in seed size, with the tall Duclair background lines showing a statistically significant increase of 2.6% ($p = 0.01$). Most other *Rht-A1* mutations contributed towards a seed size decrease, 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 3.2).

Table 3.2. Full density field trial data averaged across 2023 and 2024 environments. The *Rht-A1*-E53K mutation had the largest effect on plant height and grain yield. *Significant at the .05 probability level, **Significant at the .01 probability level. [a]Semidwarf lines contain the *Rht-B1b* allele. [b]Tall lines contain the *Rht-B1a* allele.

Tested *Rht-A1* Allele	Variety	*Rht-B1*	*Rht-A1*	Height	Grain Yield	Grain Protein	Single Grain Weight
				cm	kg ha^{-1}	g kg^{-1}	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

Figure 3.3. Plant height effect of *Rht-A1* mutant alleles in different backgrounds. The *Rht-A1*-E53K mutation had the largest effect on height. Average heights of all lines tested in full density field experiments in 2023 and 2024. Significantly different pairwise comparisons are noted: *Significant at the .05 probability level, **Significant at the .01 probability level.



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 semidwarfing 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 semidwarfing effect. These results show that binding ability of mutated RHT-1 proteins with GID1 when tested in Y2H assays may not be an accurate predictor of plant height in field conditions.

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

towards reducing height, they did not impact height, yield, or seed metrics to the same degree as *Rht-A1*-E63K. These results reinforce research showing that this specific location is especially important in the function of *Rht-1*. 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 gain of function traits that we see in semidwarf wheat cultivars. Additionally, these results 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, VHYNP, 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).

Conclusion

The *Rht-A1*-E63K mutation increased grain yield when tested in tall backgrounds without significantly decreasing grain protein content. 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 this 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 wildtype (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.

References

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.,…Gooding, M. (2016). Reduced Height (*Rht*) Alleles Affect Wheat Grain Quality. *Plos One*, *11*(5). 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

Flintham, J. E., Borner, 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.,…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., Carlson, G., Lamb, P., Nash, D., Wichman, D., Kephart, K.,…Eckhoff, J. (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., Martin, J., Stougaard, R., Guillen-Portal, F., Blake, N., Sherman, J.,…Carlson, G. (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

Lanning, S. P., Carlson, G. R., Nash, D., Wichman, D. M., Kephart, K. D., Stougaard, R. N.,…Talbert, L. E. (2006). Registration of 'Vida' wheat. *Crop Science*, *46*(5), 2315-2316. https://doi.org/10.2135/cropsci2006.03.0167

Li, A., Yang, W., Lou, X., Liu, D., Sun, J., Guo, X.,…Ling, H. Q. (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.,…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*. https://doi.org/10.1186/s12870-018-1465-4

NCEI. (2025). Daily summaries station details. Daily Summaries Station Details: BOZEMAN 6 W EXPERIMENTAL FARM, MT US, GHCND:USC00241047 | Climate Data Online (CDO) | National Climatic Data Center (NCDC). https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00241047/detail

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

Pearce, S., Saville, R., Vaughan, S. P., Chandler, P. M., Wilhelm, E. P., Sparks, C. A.,…Phillips, A. L. (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.,…Pelica, F. (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

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

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M.,…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

Zentella, R., Zhang, Z. L., Park, M., Thomas, S. G., Endo, A., Murase, K.,…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. Y., Xiong, H. C., Fu, M. Y., Guo, H. J., Zhao, L. S., Xie, Y. D.,…Liu, L. X. (2023). Genetic mapping and identification of *Rht8-B1* that regulates plant height in wheat. *Bmc Plant Biology*, *23*(1).

CHAPTER FOUR

EVALUATION OF *REDUCED HEIGHT-1 RHT-B1B*-E529K, *RHT-A1*-S50F, AND *RHT-A1*-L358F IN DURUM WHEAT

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Contributions: conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing—review and editing.

Manuscript Information

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Abstract

The wheat *(Triticum spp.) Reduced height-1* (*Rht-1*) *B1b* and *D1b* semidwarfing alleles have been well studied since their incorporation into modern breeding populations in the 1960s. However, the underlying mechanisms of how these alleles reduce plant height has not been well understood until recently. These specifics have been studied in hexaploid wheat (*Triticum aestivum* L.), but less so in tetraploid durum wheat *(Triticum turgidum* L. subsp. *durum)*. Most durum varieties are either tall or semidwarf, containing either the *Rht-B1a* (tall) or *Rht-B1b* (semidwarf) alleles. Because of the dosage dependent nature of these alleles, tetraploid semidwarf durum wheat is significantly shorter than hexaploid semidwarf wheat, meaning that in low resource environments, semidwarf durum varieties may yield less and are often too short to be effectively harvested. Thus, identification of *Rht-1* alleles that confer intermediate height is needed. Near isogenic lines were created to test previously characterized alleles *Rht-B1b*-E529K, *Rht-A1*-S50F*,* and *Rht-A1*-L358F in field trials to measure grain yield. The *Rht-B1b*-E529K allele was additionally tested in the greenhouse to evaluate differences in emergence rates at different planting depths. While *Rht-A1*-S50F*,* and *Rht-A1*-L358F affect height, the difference is slight and these alleles may reduce yield. *Rht-B1b*-E529K confers intermediate height, seed morphology, and seedling emergence when compared to lines containing the *Rht-B1a* allele or the *Rht-B1b* allele. *Rht-B1b*-E529K also increased yield compared to *Rht-B1b* in one of the five tested environments. These results show that the *Rht-B1b*-E529K allele could be useful in regions where the drastic decrease in height and reduction in seedling emergence conferred by *Rht-B1b* can be detrimental.

Introduction

Durum wheat *(Triticum turgidum* L. subsp. *durum)* is a tetraploid wheat species primarily grown for milling into semolina flour which is used to make pasta and couscous. In the United States, North Dakota and Montana are the top two producers of durum wheat where 801,277 hectares were planted in 2024 (USDA, 2025). Throughout the history of modern wheat *(Triticum spp.)* breeding, durum wheat height optimization for individual environments has been a top priority. Gain of function *Reduced Height-1* alleles, *Rht-B1b* and *Rht-D1b*, were discovered during the Green Revolution and cause a semidwarf phenotype. Thanks to these alleles, the physical height of hexaploid wheat (*Triticum aestivum* L.) varieties has been well optimized for a range of growing conditions throughout North America. Shorter varieties are more able to withstand lodging, especially under intensively managed environments with high levels of nitrogen fertilizer (Hedden, 2003; Khush, 2001; J. Peng et al., 1999). The *Rht-B1b* allele has also been introgressed into tetraploid durum wheat cultivars, but since this gene has a dosage dependent effect relative to the number of *Rht-1* homoeologs present, these alleles have a stronger effect in durum, resulting in much shorter semidwarf varieties. These semidwarf durum varieties are perfect for cultivation in southwestern deserts of the United States of America under intensive irrigation, fertilization, and sunlight, but can sometimes be too short for non-irrigated growing regions in the Northern Great Plains. As a result, most durum varieties grown in the Northern Great Plains have a tall phenotype, without the benefits that come with reduced height (Beres et al., 2020). This study looks more closely at different alleles of *Rht-1* in durum wheat that could result in an “intermediate height” phenotype, somewhere between semidwarf and tall.

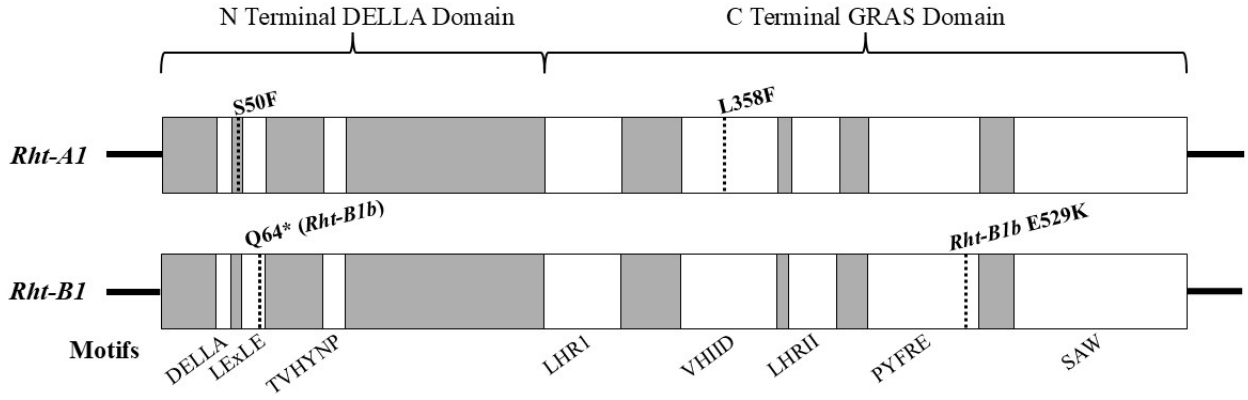
These alternative alleles could prove useful for integration into durum breeding programs in the Northern Great Plains.

Rht-1 is a well-studied DELLA protein encoding gene. DELLA is an abbreviation for a conserved region towards the beginning of the coding sequence (Asp-Glu-Leu-Leu-Ala). The molecular characteristics of DELLA protein encoding genes, including *Rht-1* in wheat, have been well-described. DELLA proteins act as negative regulators of gibberellic acid (Zentella et al., 2007). Gibberellic acid (GA3) is a phytohormone responsible for regulating seed germination, stem elongation, and other developmental processes (Hauvermale et al., 2012). In the presence of GA3, DELLA proteins are bound by GA3-Insensitive Dwarf 1 (GID1) which is a GA3 receptor protein (Shimada et al., 2008). They are then polyubiquinated by the E3 ubiquitin ligase complex which is then degraded by the 26S proteasome (Griffiths et al., 2007; Lou et al., 2016; Ueguchi-Tanaka et al., 2005). DELLA protein degradation causes the plant to respond to the GA3 signal and grow taller. If GA3 is absent, DELLA proteins are not degraded, so GA3 responses and consequently plant growth are repressed. In wheat, *Rht-1* is responsible for encoding DELLA proteins. There is a single functional copy of *Rht-1* on each of the group four chromosomes in wheat. In durum wheat, this includes one copy on the A genome, *Rht-A1*, and one copy on the B genome, *Rht-B1* (Ellis et al., 2002; Gale et al., 1975; McVittie et al., 1978; Sourdille et al., 1998).

DELLA protein encoding genes contain two domains, the N terminal DELLA domain, and the C-terminal GRAS domain. The N terminal DELLA domain is characterized by the DELLA motif which is specifically involved in the interaction with GID1, the TVHYNP motif, and LExLE motif (Ikeda et al., 2001; Itoh et al., 2002; Murase et al., 2008; Silverstone & Sun, 2000). The C-terminal GRAS domain is characterized by two leucine heptad repeat motifs

(LHRI and LHRII), the VHIID, PFYRE, and the SAW motifs (Figure 4.1). The N terminal DELLA domain plays a primary role in binding DELLA proteins to be degraded by the ubiquitin ligase complex (Bolle, 2004; Dill et al., 2004; Murase et al., 2008).

Figure 4.1. Graphical representation of the *Rht-A1* and *Rht-B1* genes, including conserved regions, motifs, and locations of point mutations discussed in this study.



The naturally occurring, dominant acting, semidwarfing, mutant alleles *Rht-B1b* and *Rht-D1b* contain nonsense mutations in the DELLA domain that dramatically alter plant growth and development. A premature stop codon in the N terminal of the DELLA domain that prevents the GID1 receptor from binding (M. Peng et al., 1999). After the stop codon within the LExLE motif in *Rht-B1b* and *Rht-D1b*, *Rht-1* translation reinitiates resulting in N terminally truncated DELLA proteins (Van De Velde et al., 2021). Since the DELLA proteins are shortened, they cannot detect GA3 signals mediated by GID1 rendering the plant partially insensitive to GA3 resulting in a semidwarf phenotype. Translation reinitiation only occurs in certain tissues. Truncated DELLA proteins were detected in the stems and spikes of semidwarf wheat, but not in the seeds, potentially explaining why the GA3 response in *Rht-B1b* or *Rht-D1b* genotype varieties is

preserved in seeds (seed dormancy is not affected) but GA3 response in stems is decreased (Flintham et al., 1997; Hedden, 2003; Hoogendoorn et al., 1990; 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 et al., 1981). Furthermore, decreasing stem height allowed for more assimilate partitioning into developing heads which increases harvest index (Youssefian et al., 1992). This has historically resulted in yield increases of about 20 % (Flintham et al., 1997). Either *Rht-B1b* or *Rht-D1b* are now found in at least 70 % of all modern hexaploid wheat cultivars (Evans, 1998). 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 & Qualset, 1976; Rebetzke et al., 2007; Schillinger et al., 1998). *Rht-B1b* and *Rht-D1b* also reduce seed protein content and size (Appleford et al., 2007; Casebow et al., 2016).

Other mutations in *Rht-1* and its orthologs have been extensively studied in wheat and other plants including *Arabidopsis thaliana* L., maize *(Zea mays* L. subsp. *mays)*, barley (*Hordeum vulgare* L,), and rice (*Oryza sativa* L.) (Foster, 1977; Fujioka et al., 1988; Harberd & Freeling, 1989; Ikeda et al., 2001; Koorneef et al., 1985; Peng et al., 1997). Missense mutations in the N terminal region of *Rht-1* usually cause a decrease in height. This is a result of decreased ability of the DELLA protein to bind with GID1, resulting in repression of GA responses even when GA is present (Chandler et al., 2008; Liu et al., 2010; J. Peng et al., 1999). Missense and nonsense mutations in the GRAS domain of *Rht-1* genes generally cause an increase in height.

This is the result of the mutated DELLA protein having reduced or negated ability to repress GA3 responses (Chandler & Harding, 2013; Thomas, 2017; Ugrin et al., 2023).

Current *Rht-1* mutant alleles impose greater challenges in tetraploid wheat compared to hexaploid wheat. Because durum is a tetraploid with an A and B genome but no D genome, there is a greater overall dosage response from a mutation in the B genome resulting in compounding pleiotropic effects of *Rht-B1b.* Because of this, plant height, seed protein content, and seed size reductions in semidwarf varieties (when compared to tall varieties) are greater in durum wheat than in hexaploid bread wheat (Mathews et al., 2006). A mutant *Rht-B1b* allele in semidwarf hexaploid wheat results in a height reduction of about 21 %, while the same mutant *Rht-B1b* allele in semidwarf tetraploid durum wheat results in a height decrease of up to 45 % (Flintham et al., 1997; Hoogendoorn et al., 1990; Mathews et al., 2006). This level of height reduction in durum wheat can result in plants too short for effective mechanical harvest in low moisture, rainfed environments. *Rht-B1b* also more drastically reduces coleoptile length in durum (Pandey et al., 2015; Trethowan et al., 2001). If coleoptiles are short, poor emergence may occur in dryland environments where crops are seeded deeper in order to access available moisture (Jobson et al., 2021; Schillinger et al., 1998). Due to these drawbacks, the vast majority of durum varieties grown in Montana, North Dakota, and Saskatchewan have a standard height phenotype and contain the *Rht-B1a* allele (Beres et al., 2020).

Other novel *Rht-B1* alleles have been researched and described in durum wheat in order to create more options for breeders other than *Rht-B1a* and *Rht-B1b* (Zhou et al., 2023). One promising allele is *Rht-B1b*-E529K, which contains a novel mutation within the *Rht-B1b* allele that increased plant height by 21 % in relation to *Rht-B1b,* indicating the potential for an

intermediate height phenotype in durum (Mo et al., 2018). Brown et al. (2022) showed that the *Rht-B1b*-E529K mutation significantly increased coleoptile length relative to *Rht-B1b* and decreased length compared to *Rht-B1a*. Additionally, the effect of the *Rht-B1b*-E529K mutation was greater in the presence of GA3 compared to water. These findings were consistent with other studies that cite *Rht-B1b* and *Rht-D1b* as being the only *Rht-1* alleles that impact coleoptile length (Fick & Qualset, 1976; Jobson et al., 2021; Liatukas & Ruzgas, 2011; Schillinger et al., 1998).

Previously, no *Rht-A1* mutations had been found to significantly decrease height in Durum. 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. Brown et al. (2022) described the creation of two *Rht-A1* missense mutations *Rht-A1*-S50F and *Rht-A1*-L358F. The *Rht-A1*-S50F mutation occurs directly between the conserved DELLA and LExLE motifs, and the *Rht-A1*-L358F mutation occurs in the conserved VHIID motif (Figure 4.1).

Brown et al. (2022) tested *Rht-A1*-S50F and *Rht-A1*-L358F along with *Rht-B1b*-E529K in vitro in a yeast two-hybrid assay, and in single spaced plant field trials. The results of the yeast two-hybrid assay showed that *Rht-A1a* and *Rht-B1a* interacted with GID1 in the presence and absence of GA3 in vitro. The *Rht-A1*-S50F missense mutation showed no interaction in the absence of GA3 but did interact to a similar degree as *Rht-B1a* in the presence of GA3. *Rht-A1*-L358F showed a weak interaction in the absence of GA3 and normal binding to GID1 in the presence of GA3. *Rht-A1* 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 documented spaced-plant conditions (Brown et al., 2022). These results are limited, as the spaced-plant trials are not representative of typical field practices in which plants are seeded at much higher density. Furthermore, the plants used in these previous trials were not near-isogenic lines and therefore other unknown genetic interactions could have influenced results.

This study considers the effects of two *Rht-A1* alleles *Rht-A1*-S50F and *Rht-A1*-L358F along with the *Rht-B1* allele *Rht-B1b*-E529K in near-isogenic line (NIL) populations. These NIL were grown in commercial seeding rate trials to test the effect of the three alleles on plant height, grain yield, single grain weight, and grain protein content, to determine their suitability for introgression into durum wheat breeding programs. NIL testing the *Rht-B1b*-E529K allele were also grown in a greenhouse-based emergence trial to show the effect of intermediate coleoptile length on seedling emergence.

Materials and Methods

Plant Material

The *Rht-B1b*-E529K allele was originally derived from a mutant Targeting Induced Local Lesions in Genomes (TILLING) population in the variety 'Kronos' (PI 576168) (Krasileva et al., 2017; Mo et al., 2018; Tsai et al., 2011). The *Rht-A1*-S50F and *Rht-A1*-L358F alleles were derived from an ethyl methanesulfonate (EMS) induced mutant population in the durum variety 'Divide' (PI 642021) (Brown et al., 2022; Elias & Manthey, 2007). The *Rht-B1b*-E529K portion of the experiment aims to compare *Rht-B1b*-E529K mutant allele with *Rht-B1b* allele in a semidwarf background, and *Rht-B1b*-E529K mutant allele with *RHT-B1a* allele in a tall background. Both backgrounds were fixed for *Rht-A1a* (wildtype) allele. The *Rht-A1* mutation

portions of the experiment aim to compare *Rht-A1*-S50F, *Rht-A1*-L358F alleles with *Rht-A1a* wildtype allele in both a tall background with fixed *Rht-B1a* allele, and in a semidwarf background with fixed *Rht-B1b* allele. These objectives resulted in a total of six separate experimental comparisons. The three alleles (*Rht-A1*-S50F, *Rht-A1*-L358F, and *Rht-B1b*-E529K) were crossed into two different recurrent parents, 'Lustre' (PI 695072), a standard height cultivar (Hogg et al., 2022) and 'MT112219', a semidwarf experimental line. This was followed by backcrossing to the recurrent parents for five generations and then selfing for two generations to create BC_5F_2 derived NIL. To determine genotypes at each generation, leaf tissue was collected from individual two-week-old seedlings and DNA was extracted as previously described by Riede & Anderson (1996). The DNA was used in PCR reactions using the nested approach of Li et al. (2013) utilizing *Rht-1* A or B genome-specific primers to amplify the *Rht-A1* or *Rht-B1* coding sequence, followed by amplification of the region containing the *Rht-A1*-S50F and *Rht-A1*-L358F mutations using internal primers. 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) and the sequence was compared to the *Rht-A1* reference accession JF930277.1 and the *Rht-B1* reference accession, JF930278.1 (www.ncbi.nlm.nih.gov) using the DNASTAR SeqMan Pro software (DNASTAR version 15.0.1.1, DNASTAR Inc. Madison, WI). In the last backcross generation, single heterozygous plants representing each of the six comparisons were allowed to self–pollinate and BC_5F_2 plants homozygous for the alternative alleles were selected. Each selected plant provided $BC_5F_{2:3}$ seed that was planted in Yuma, AZ in October 2021 for seed increase. $BC_5F_{2:4}$ seed was used to plant field trials in Bozeman, MT in 2022. $BC_5F_{2:5}$ seed from the 2022 Bozeman trial was used as seed in all 2023

trials. Two lines representing each genotype class within each of the six comparisons were selected as entries to be included in each experimental location and year of this study.

Experimental Design

In 2022, experiments were grown at the Arthur H. Post Agronomy farm near Bozeman, MT in an irrigated environment and in a rainfed environment. In 2023, experiments were grown at the Montana State University Northern Agricultural Research Center near Havre, MT, at the Eastern Agricultural Research Center near Sidney, MT, and again at the Arthur H. Post Agronomy Farm near Bozeman, MT, all in a rainfed environment. In each environment, lines from the two different recurrent parent backgrounds were grown in separate but adjacent experiments. Within each of these two sub experiments, plots were organized in a randomized complete block split plot design, with compared allele as the main plot (*Rht-B1b*-E529K*, Rht-A1*-L358F*,* and *Rht-A1*-S50F*)* and genotype as the sub plot (mutant or wildtype). Two replicates were grown in each environment, except for the 2023 Bozeman rainfed environment, which included four replicates.

Field conditions

At the Post Agronomy Farm, plots consisted of four, three meter long rows spaced 30 cm apart. At the Post Farm in 2022, seeds were mechanically sown to a depth of five centimeters on 17 May. The irrigated experiment received five centimeters of water applied using hand line sprinklers on 21 June, 26 June, and 2 July. For weed and disease control, 0.04 l/ha of Affinity® TankMix (DuPont de Nemours and Co. Wilmington, DE USA), 0.50 l/ha MCPE (Agrilliance, LLC. St. Paul MN USA), 1.17 l/ha of Discover® (Syngenta Crop Protection LLC. Greensboro, NC USA) were applied for weed and disease control. From 1 May to 31 August 2022, the Arthur

H Post Agronomy Farm received 19.8 cm of precipitation. 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 (NCEI, 2025a). In 2023 at the Post Agronomy Farm, seeds were mechanically sown to a depth of five centimeters on 3 and 4 May. For weed and disease control, 0.04 l/ha of Affinity® Tank Mix (DuPont de Nemours and Co. Wilmington, DE USA), 1.2 l/ha of OpenSky® (Corteva Agriscience LLC, Indianapolis, IN USA), 0.3 l/ha of Alto® 100SL (Syngenta Crop Protection LLC. Greensboro, NC USA), and 0.4 l/ha of 2-4D LV6 were applied. From 1 May to 31 August 2023, the Post Agronomy Farm received 21.9 cm of precipitation. No supplemental irrigation water was provided in 2023. The highest recorded air temperature was 38.3 °C on 24 July 2023 and the lowest recorded air temperature was 0.5 ° C on 21 June 2023 (NCEI, 2025a).

At the Northern Agricultural Research Center near Havre, MT in 2023, plots consisted of three, 5.5 m rows spaced 30 cm apart. Seeds were sown on 29 April to a depth of four centimeters. From 1 May to 31 August, this site received 17.0 cm of precipitation. The highest recorded air temperature was 38.9 °C on 25 July and the lowest recorded air temperature was 1.7 °C on 21 June (NCEI, 2025b).

At the Eastern Agricultural Research Center near Sidney, MT in 2023, plots consisted of five, three meter rows spaced 22 cm apart. Seeds were sown on 26 April 2023. For weed and disease control, 1.2 l/ha of OpenSky® (Corteva Agriscience LLC, Indianapolis, IN USA) was applied. From 1 May to 31 August, this location received 22.4 cm of precipitation. The highest recorded air temperature was 40.6 °C on 25 July and the lowest recorded air temperature was -4.4 °C on 24 May (NCEI, 2025c).

Field Measurements

Plant height was measured at physiological maturity as the distance from the soil surface to the terminal spikelet of a representative primary tiller, not including awns. Plots were combine harvested, and grain yield from each plot was weighed prior to cleaning. Grain protein was measured by near-infrared transmittance using a Foss Infratec 1241 Grain Analyzer (Foss North America, Silver Springs, MD) and individual seed weight was calculated by weighing a sample of 200 manually counted seeds.

Greenhouse Emergence Trial

Four total NIL representing the *Rht-B1b*-E529K vs *Rht-B1a* genotype comparison and the *Rht-B1b*-E529K vs *Rht-B1b* comparison were randomly selected from the field-based experiment to test for emergence between *Rht-B1b*-E529K lines when compared to both tall and semidwarf lines. Seed viability was examined using a germination test to ensure consistency across genotypes. To test germination rates, 50 seeds per seed lot were surface sterilized and germinated for seven days in moist, sterile germination paper. All seed lots displayed a germination rate of ≥ 96 %. An eight inch (203 mm) diameter sieve with No. 7 (2.8 mm) openings was used to remove small seeds from seed lots to ensure uniform seed size across the experiment. Seedlings were grown in a greenhouse at the Montana State University-Bozeman Plant Growth Center in 25 cm diameter pots. Sunshine® mix soil was used as a growth medium (Sun Gro Horticulture, Agawam, MA, USA). Temperature settings were maintained at 22 °C during the day and 14 °C at night. The experiment tested seed emergence across four planting depths: 5 cm, 7.5 cm, 10 cm, and 15 cm. For each depth, 20 seeds of the same genotype were planted in a single pot, with all seeds planted at the same depth. The study included four genotypes, four planting depths, and

five replicates per combination, resulting in a total of 80 pots. Equal soil volumes were added to each pot to ensure uniform soil conditions. Following planting, soil moisture in the pots was brought to saturation. Pots were watered one other time, seven days after planting, to maintain adequate moisture levels throughout the experiment. No fertilizer was used. Pots were arranged in a randomized complete block design to account for any temperature variability in the greenhouse. A daily record was kept of how many seedlings had emerged from the surface of the soil within each pot. Data was collected for a total of 10 days after planting. The first emerged seedling was observed on day five. Emergence rates based on the number of emerged seedlings in each pot were calculated for each day, and these rates were used as datapoints for statistical analysis.

Statistical Analysis

Data from all experiments in this study was analyzed with the lme4 package (Bates et al., 2015) in R (R Foundation for Statistical Computing. Version 4.0.5. Vienna, Austria). For field experiments, statistical analyses were performed using the mean values from the two lines that represented each genotype class within each allele comparison, as in Lanning et al. (2012). Each location and year combination was treated as an individual environment. The tall (Lustre) and semidwarf (MT112219) backgrounds were analyzed separately. Each background was analyzed using a mixed linear model for a randomized block split plot design combined over environments. The model included environment replication within environments, allele group (*Rht-B1b*-E529K*, Rht-A1*-L358F*,* and *Rht-A1*-S50F*)*, replication x allele group within environment (main plot error), genotype (wild type or mutant), and allele group x genotype. Comparisons were made between genotype classes within each allele group. For the greenhouse-

based emergence trial, the tall (Lustre) and semidwarf (MT112219) genetic backgrounds were analyzed using a mixed linear model structured as a split-plot design. Genotype and planting depth were treated as the main-plot factors, while day was analyzed as a subplot factor to account for repeated measurements over time. The model included genotype, planting depth, and day, along with their interactions, as fixed effects. Block was included as a random effect, with an additional random term for the genotype-by-depth interaction within each block to account for the split-plot structure. Emergence rates for each pot on each day were treated as individual data points. Pairwise comparisons were conducted to assess statistical significance within each genotype, depth, and day combination.

Results and Discussion

Field Experiment Results

Rht-B1b-E529K allele showed an effect similar to that of a standard *Rht-B1b* allele but at a much lesser magnitude (Table 4.1). When compared to *Rht-B1a*, height was reduced by 4.3 cm ($p < 0.001$). When compared to *Rht-B1b*, height was increased by an average of 21 cm ($p < 0.001$), single grain weight was increased by 3.0 mg ($p < 0.001$), and grain protein content was increased by 3.0 g kg^{-1} ($p < 0.001$). In the Bozeman 2023 rainfed environment there was a significant increase in grain yield of 8.8 % ($p = 0.048$) conferred from the *Rht-B1b*-E529K genotype when comparing *Rht-B1b* genotypes to *Rht-B1b*-E529K genotypes in a semidwarf background, but there were no differences between these two genotypes in other environments, resulting in a significant genotype by environment interaction for this comparison (Table 4.1, See supplemental data for mean yields within each environment). Average grain yield was not changed in any of the tested environments when comparing *Rht-B1a* genotypes to *Rht-B1b*-

E529K genotypes in a tall background. The *Rht-B1b*-E529K allele affected height in the same direction in each environment, but at different magnitudes in different environments in the semidwarf background comparison, resulting in some significant genotype by environment interaction. Results from this study consistently confirm the intermediate height, seed weight, and protein content phenotype of *Rht-B1b-E529K* lines when compared to tall *Rht-B1a* and semidwarf *Rht-B1b* genotype lines (Table 4.1 and supplemental data).

Table 4.1. The impact of *Rht-1* durum wheat mutations averaged over five Montana environments (location-year combinations). The *Rht-B1b*-E529K allele had the greatest impact on measured parameters, displaying intermediate phenotype between tall *(Rht-B1a)* and semidwarf *(Rht-B1b)* lines. *Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level. [a] Denotes a comparison with significant GxE interaction (see supplemental data tables for data broken down by location)

Tested Allele	**Back-ground**	**Genotype**	**Plant Height**	**Grain Yield**	**Single Grain Weight**	**Grain Protein Content**
			Cm	kg Ha^{-1}	mg	g kg^{-1}
Rht-B1b-E529K	*Rht-B1a* (Lustre)	mutant	82.9±1.2***	4368±220	34.2±0.85***[a]	147±2.7[a]
		wildtype	87.8±1.2	4370±220	35.8±0.86	147±2.7
	Rht-B1b (MT112219)	mutant	88.4±2.1***[a]	4085±123[a]	40.3±0.72***[a]	143±2.5*[a]
		wildtype	70.8±1.8	4102±117	36.7±0.73	140±2.3
Rht-A1-L358F	*Rht-B1a* (Lustre)	mutant	90.1±0.8	4447±78.6	37.3±0.73	148±1.9
		wildtype	91.4±0.8	4337±78.6	37.2±0.73	148±1.9
	Rht-B1b (MT112219)	mutant	65.2±0.5	4186±129	36.6±0.80	138±1.7
		wildtype	64.4±0.5	4296±134	36.1±0.82	137±1.9
Rht-A1-S50F	*Rht-B1a* (Lustre)	mutant	90.1±0.6***	4139±146	38.3±0.79	148±1.4
		wildtype	92.3±0.6	4130±146	38.8±0.79	149±1.4
	Rht-B1b (MT112219)	mutant	67.1±0.7*	3800±192	36.4±0.72	144±2.6*
		wildtype	65.3±0.7	4144±188	36.5±0.72	138±2.5

In a tall background, the *Rht-A1*-L358F allele decreased height by an average of 1.7 cm ($p = 0.03$). In a semidwarf background, the *Rht-A1*-L358F allele had no effect on height but did

increase protein content by an average of 0.4 % ($p < 0.01$). Similarly, The *Rht-A1*-S50F allele caused an average height decrease of 1.7 cm ($p = 0.03$) in the tall background, but no significant height difference in the semidwarf background. There was a trend toward increased protein (0.3 %, $p = 0.06$). These differences were consistent across all environments, with no significant genotype by environment interactions. No other differences were detected (Table 4.1 and supplemental data).

Greenhouse Emergence Trial Results

The *Rht-B1b*-E529K allele again displayed an intermediate phenotype in the emergence trial. When compared to *Rht-B1b* in a semidwarf background, the *Rht-B1b*-E529K genotype seedlings emerged sooner when planted at depths of 5 cm and 7.5 cm and emerged at higher rates overall when planted at depths of 10 cm and 15 cm (Figure 4.2). When compared to *Rht-B1a* in a tall background, the *Rht-B1b*-E529K genotype seedlings emerged similarly at depths of 5 cm and 7.5 cm but emerged at a lower rate overall at 10 cm and 15 cm planting depths (Figure 4.3). These results show that at shallow planting depths, the *Rht-B1b*-E529K allele confers a slight advantage in emergence time over the *Rht-B1b* allele and no disadvantage when compared to the *Rht-B1a* allele. At deeper planting depths, the *Rht-B1b*-E529K allele confers a significant overall emergence advantage over the *Rht-B1b* allele, although emergence rates are not brought up to the same level as seedlings with the *Rht-B1a* allele.

Figure 4.2. Seedling emergence results from the MT112219 (semidwarf) background over time at four different seeding depths, 5 cm, 7.5 cm, 10 cm, and 15 cm. Relative to a semidwarf (*Rht-B1b*) genotype, *Rht-B1b*-E529K genotype seedlings emerged significantly sooner at 5, 7.5 and 10 cm planting depths, and emerged overall at a higher rate at the 15 cm planting depth.

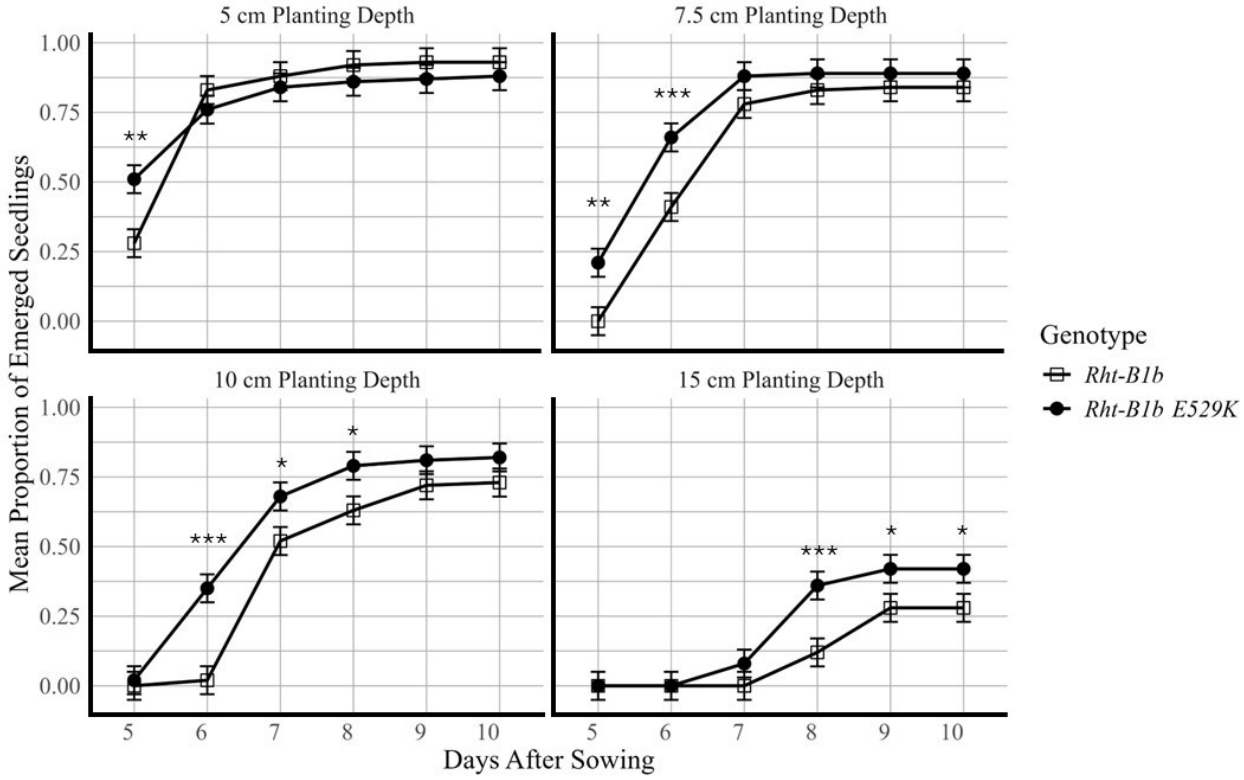
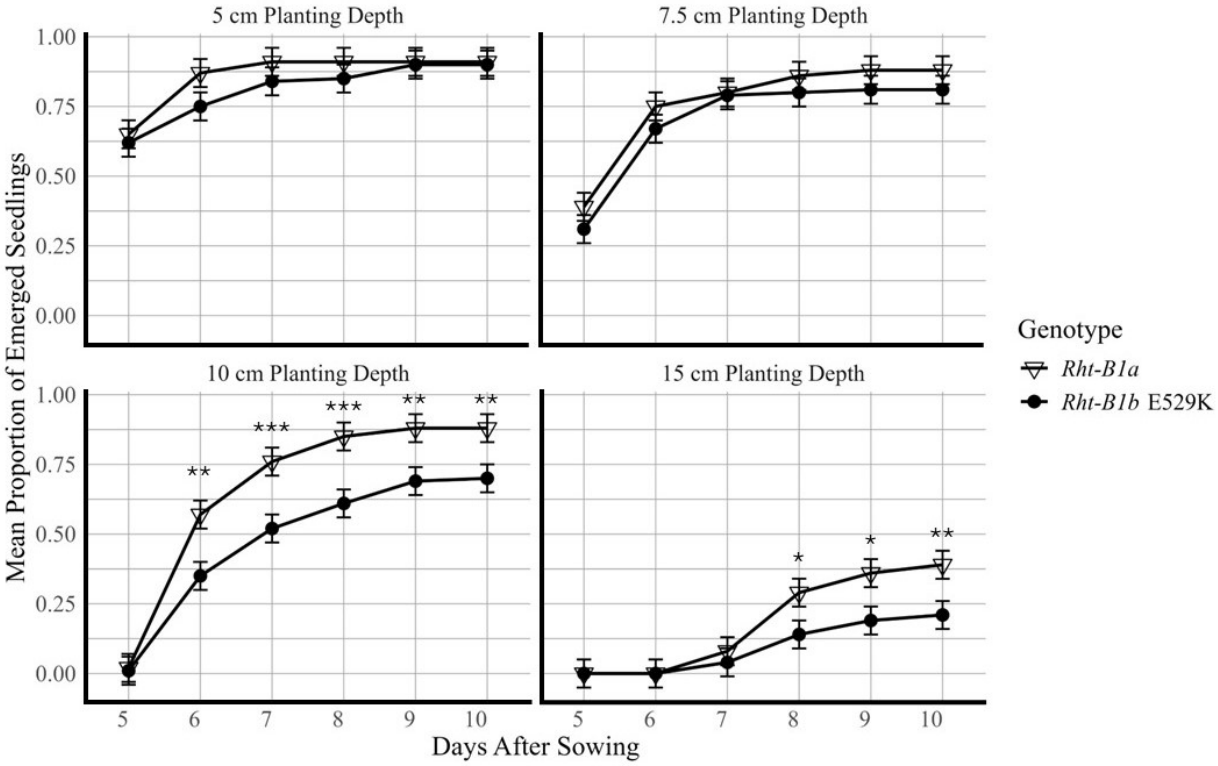


Figure 4.3. Seedling emergence results from the Lustre (tall) background over time at four different seeding depths, 5 cm, 7.5 cm, 10 cm, and 15 cm. Relative to a standard height (*Rht-B1a*) genotype, *Rht-B1b*-E529K seedlings emerged similarly at 5 cm and 7.5 cm seeding depths but emerged at a significantly lower rate overall at 10 cm and 15 cm seeding depths.



Discussion

The results of experiments testing *Rht-B1b*-E529K are consistent with Mo et al. (2018) and with the single plant experiments of Brown et al. (2022). Relative to *Rht-B1b, Rht-B1b*-E529K increased height by 32 %, and when compared to *Rht-B1a, Rht-B1b*-E529K decreases height by five percent, however, these results are most likely background dependent, as the effects of the *Rht-B1b* allele are known to differ among varieties (Hoogendoorn et al., 1990; Mathews et al., 2006). These results support the idea that a missense mutation within the GRAS domain of the *Rht-B1b* allele decreases the gain of function, semi dwarfing effect that *Rht-B1b* has on plant growth and seed traits. The protein that results from re-initiation of translation in

Rht-B1b is normally able to partially repress GA function. However, since this reinitiated portion contains the E529K mutation, its ability to repress GA function is reduced, resulting in an intermediate phenotype (Mo et al., 2018; Van De Velde et al., 2021). Given that the *Rht-B1b*-E529K allele confers significant yield gain compared with semidwarf genotype in one of the five tested environments, no yield loss in any of the other environments, increased emergence rate compared with semidwarf genotype, no significant single grain weight reductions, and no significant protein content reductions, these results support the introgression of this allele into durum breeding programs in the Northern Great Plains. These results add to previous evidence that mid-height wheat varieties can have a yield advantage in drought prone environments (Mathews et al., 2006; Richards, 1992).

This study found that the *Rht-A1*-L358F and *Rht-A1*-S50F alleles caused a height decrease in a tall background, but no significant change in a semidwarf background. This decrease is consistent with studies that show that mutations in the N terminal of DELLA protein encoding genes are associated with a reduction in height due to decreased ability to bind with GID1 (Chandler et al., 2008; Liu et al., 2010; J. Peng et al., 1999). There was an increase in protein content in lines containing these alleles in a semidwarf background, potentially correlated with a slight reduction in grain yield, which would be consistent with documented negative correlations between protein content and grain yield (Joppa et al., 1997; Tabbita et al., 2017). These results are also consistent with results from the Brown et al. (2022) single plant study.

Conclusion

These results confirm that missense mutations of *Rht-A1* in durum wheat do have a small but detectable phenotypic effect on plant height in durum wheat when grown at commercial

seeding rates. Results show support for the incorporation of *Rht-B1b*-E529K allele into durum breeding programs in the Northern Great Plains as it practically incorporates benefits of both tall and semidwarf varieties in a single intermediate height phenotype.

Appendix Material

See supplemental table B4.1 for field experiment data broken down by environment and year.

References

Appleford, N. E. J., Wilkinson, M. D., Ma, Q., Evans, D. J., Stone, M. C., Pearce, S. P.,…Lenton, J. R. (2007). Decreased shoot stature and grain α-amylase activity following ectopic expression of a gibberellin 2-oxidase gene in transgenic wheat. *Journal of Experimental Botany*, *58*(12), 3213-3226. https://doi.org/10.1093/jxb/erm166

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

Beres, B. L., Rahmani, E., Clarke, J. M., Grassini, P., Pozniak, C. J., Geddes, C. M.,…Ransom, J. K. (2020). A Systematic Review of Durum Wheat: Enhancing Production Systems by Exploring Genotype, Environment, and Management (G x E x M) Synergies. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.568657

Bolle, C. (2004). The role of GRAS proteins in plant signal transduction and development. *Planta*, *218*, 683-692. https://doi.org/10.1007/s00425-004-1203-z

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.,…Gooding, M. (2016). Reduced Height (*Rht*) Alleles Affect Wheat Grain Quality. *Plos One*, *11*(5). 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

Chandler, P. M., Harding, C. A., Ashton, A. R., Mulcair, M. D., Dixon, N. E., & Mander, L. N. (2008). Characterization of gibberellin receptor mutants of barley (*Hordeum vulgare* L.). *Molecular Plant*, *1*(2), 285-294. https://doi.org/10.1093/mp/ssn002

Dill, A., Thomas, S. G., Hu, J. H., Steber, C. M., & Sun, T. P. (2004). The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell*, *16*(6), 1392-1405. https://doi.org/10.1105/tpc.020958

Elias, E. M., & Manthey, F. A. (2007). Registration of 'Divide' Durum Wheat. *Journal of Plant Registrations*, *1*(1), 7-8. https://doi.org/10.3198/jpr2006.05.0281crc

Ellis, M. H., Spielmeyer, W., Gale, K. R., Rebetzke, G. J., & Richards, R. A. (2002). "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theoretical and Applied Genetics*, *105*(6-7), 1038-1042. https://doi.org/10.1007/s00122-002-1048-4

Evans, L. T. (1998). *Feeding the Ten Billion: Plants and Population Growth*. Cambridge University Press.

Fick, G. N., & Qualset, C. O. (1976). Seedling Emergence, Coleoptile Length, and Plant Height Relationships in Crosses of Dwarf and Standard-Height Wheats. *Euphytica*, *25*(3), 679-684. https://doi.org/10.1007/BF00041606

Flintham, J. E., Borner, 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

Foster, C. A. (1977). Slender: An accelerated extension growth mutant of barley. Barley Genetics Newsletter, 7(24–27).

Fujioka, S., Yamane, H., Spray, C. R., Katsumi, M., Phinney, B. O., Gaskin, P.,…Takahashi, N. (1988). The dominant non-gibberellin-responding dwarf mutant (D8) of maize accumulates native gibberellins. *Proceedings of the National Academy of Sciences*, *85*(23), 9031-9035. https://doi.org/10.1073/pnas.85.23.9031

Gale, M. D., Law, C. N., & Worland, A. J. (1975). Chromosomal Location of a Major Dwarfing Gene from Norin 10 in New British Semi-Dwarf Wheats. *Heredity*, *35*(Dec), 417-421. https://doi.org/10.1038/hdy.1975.112

Gale, M. D., Marshall, G. A., & Rao, M. V. (1981). A Classification of the Norin 10 and Tom Thumb Dwarfing Genes in British, Mexican, Indian and Other Hexaploid Bread Wheat-Varieties. *Euphytica*, *30*(2), 355-361. https://doi.org/10.1007/BF00033997

Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z. L., Powers, S. J.,…Thomas, S. G. (2007). Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis (vol 18, pg 3399, 2006). *Plant Cell*, *19*(2), 726-726. https://doi.org/10.1105/tpc.107.190261

Harberd, N. P., & Freeling, M. (1989). Genetics of dominant gibberellin-insensitive dwarfism in maize. *Genetics*, *121*(4), 827-838. https://doi.org/10.1093/genetics/121.4.827

Hauvermale, A. L., Ariizumi, T., & Steber, C. M. (2012). Gibberellin Signaling: A Theme and Variations on DELLA Repression. *Plant Physiology*, *160*(1), 83-92. https://doi.org/10.1104/pp.112.200956

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

Hogg, A. C., Carr, P., Eberly, J., Chen, C. C., Kowatch-Carlson, C., Crutcher, F.,…Giroux, M. (2022). Registration of 'Lustre' durum wheat. *Journal of Plant Registrations*, *16*(3), 576-584. https://doi.org/10.1002/plr2.20214

Hoogendoorn, J., Rickson, J. M., & Gale, M. D. (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.,…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

Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M., & Matsuoka, M. (2002). The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. *Plant Cell*, *14*(1), 57-70. https://doi.org/10.1105/tpc.010319

Jobson, E. M., Martin, J. M., Sharrock, R., Hogg, A. C., & Giroux, M. J. (2021). Identification and molecular characterization of novel *Rht-1* alleles in hard red spring wheat. *Crop Science*, *61*(2), 1030-1037. https://doi.org/10.1002/csc2.20375

Joppa, L. R., Du, C. H., Hart, G. E., & Hareland, G. A. (1997). Mapping gene(s) for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines. *Crop Science*, *37*(5), 1586-1589. https://doi.org/10.2135/cropsci1997.0011183X003700050030x

Khush, G. S. (2001). Green revolution: the way forward. *Nature reviews genetics*, *2*(10), 815-822. https://doi.org/10.1038/35093585

Koorneef, M., Elgersma, A., Hanhart, C. v., van Loenen-Martinet, E., Van Rijn, L., & Zeevaart, J. (1985). A gibberellin insensitive mutant of Arabidopsis thaliana. *Physiologia Plantarum*, *65*(1), 33-39. https://doi.org/10.1111/j.1399-3054.1985.tb02355.x

Krasileva, K. V., Vasquez-Gross, H. A., Howell, T., Bailey, P., Paraiso, F., Clissold, L.,…Dubcovsky, J. (2017). Uncovering hidden variation in polyploid wheat. *Proceedings*

of the National Academy of Sciences of the United States of America, *114*(6), E913-E921. https://doi.org/10.1073/pnas.1619268114

Lanning, S., Martin, J., Stougaard, R., Guillen-Portal, F., Blake, N., Sherman, J.,…Carlson, G. (2012). Evaluation of near-isogenic lines for three height-reducing genes in hard red spring wheat. *Crop science*, *52*(3), 1145-1152.

Liatukas, Z., & Ruzgas, V. (2011). Coleoptile length and plant height of modern tall and semi-dwarf European winter wheat varieties. *Acta Societatis Botanicorum Poloniae*, *80*(3), 197-203. https://doi.org/10.5586/asbp.2011.018

Liu, C., Wang, J., Huang, T., Wang, F., Yuan, F., Cheng, X.,…Liu, K. (2010). A missense mutation in the VHYNP motif of a DELLA protein causes a semi-dwarf mutant phenotype in Brassica napus. *Theoretical and Applied Genetics*, *121*, 249-258. https://doi.org/10.1007/s00122-010-1306-9

Lou, X. Y., Li, X., Li, A. X., Pu, M. Y., Shoaib, M., Liu, D. C.,…Yang, W. L. (2016). Molecular Characterization of Three *GIBBERELLIN-INSENSITIVE DWARF2* Homologous Genes in Common Wheat. *Plos One*, *11*(6). https://doi.org/10.1371/journal.pone.0157642

Mathews, K. L., Chapman, S. C., Trethowan, R., Singh, R. P., Crossa, J., Pfeiffer, W.,…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

McVittie, J. A., Gale, M. D., Marshall, G. A., & Westcott, B. (1978). The intra-chromosomal mapping of the Norin 10 and Tom Thumb dwarfing genes. *Heredity*, *40*(1), 67-70. https://doi.org/10.1038/hdy.1978.8

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*. 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

NCEI (National Centers for Environmental Information). (2025). Daily summaries station details: Bozeman 6 W Experimental Farm, MT US, GHCND:USC00241047. Climate Data Online (CDO) | National Centers for Environmental Information (NCEI). https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00241047/detail

NCEI (National Centers for Environmental Information). (2025). Daily summaries station details: Havre Airport ASOS, MT US, GHCND:USW00094012. Climate Data Online (CDO) | National Centers for Environmental Information (NCEI).

https://www.ncei.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USW00094012/detail

NCEI (National Centers for Environmental Information). (2025). Daily summaries station details: Sidney, MT US, GHCND:USC00247560. Climate Data Online (CDO) | National Centers for Environmental Information (NCEI). https://www.ncei.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00247560/detail

Pandey, M., Singh, A. K., DePauw, R. M., Bokore, F. E., Ellouze, W., Knox, R. E., & Cuthbert, R. D. (2015). Coleoptile length, gibberellin sensitivity, and plant height variation of durum wheat in Canada. *Canadian Journal of Plant Science*, *95*(6), 1259-1264. https://doi.org/10.4141/cjps-2015-154

Pearce, S., Saville, R., Vaughan, S. P., Chandler, P. M., Wilhelm, E. P., Sparks, C. A.,…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.,…Pelica, F. (1999). 'Green revolution'genes encode mutant gibberellin response modulators. *nature*, *400*(6741), 256-261. https://doi.org/10.1038/22307

Peng, J. R., Carol, P., Richards, D. E., King, K. E., Cowling, R. J., Murphy, G. P., & Harberd, N. P. (1997). The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes & Development*, *11*(23), 3194-3205. https://doi.org/10.1101/gad.11.23.3194

Peng, M., Gao, M., Abdel-Aal, E. S. M., Hucl, P., & Chibbar, R. N. (1999). Separation and characterization of A- and B-type starch granules in wheat endosperm. *Cereal Chemistry*, *76*(3), 375-379. https://doi.org/10.1094/CCHEM.1999.76.3.375

Rebetzke, G. J., Ellis, M. H., Bonnett, D. G., & Richards, R. A. (2007). Molecular mapping of genes for coleoptile growth in bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *114*(7), 1173-1183. https://doi.org/10.1007/s00122-007-0509-1

Richards, R. (1992). The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. *Australian Journal of Agricultural Research*, *43*(3), 517-527. https://doi.org/10.1071/AR9920517

Riede, C. R., & Anderson, J. A. (1996). Linkage of RFLP markers to an aluminum tolerance gene in wheat. *Crop Science*, *36*(4), 905-909. https://doi.org/10.2135/cropsci1996.0011183X0036000400015x

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

Shimada, A., Ueguchi-Tanaka, M., Nakatsu, T., Nakajima, M., Naoe, Y., Ohmiya, H.,…Matsuoka, M. (2008). Structural basis for gibberellin recognition by its receptor GID1. *Nature*, *456*(7221), 520-U544. https://doi.org/10.1038/nature07546

Silverstone, A. L., & Sun, T. P. (2000). Gibberellins and the green revolution. *Trends in Plant Science*, *5*(1), 1-2. https://doi.org/Doi 10.1016/S1360-1385(99)01516-2 https://doi.org/10.1016/S1360-1385(99)01516-2

Sourdille, P., Charmet, G., Trottet, M., Tixier, M. H., Boeuf, C., Negre, S.,…Bernard, M. (1998). Linkage between RFLP molecular markers and the dwarfing genes Rht-B1 and Rht-D1 in wheat. *Hereditas*, *128*(1), 41-46. https://doi.org/10.1111/j.1601-5223.1998.00041.x

Tabbita, F., Pearce, S., & Barneix, A. J. (2017). Breeding for increased grain protein and micronutrient content in wheat: Ten years of the GPC-B1 gene. *Journal of Cereal Science*, *73*, 183-191. https://doi.org/10.1016/j.jcs.2017.01.003

Thomas, S. G. (2017). Novel *Rht-1* dwarfing genes: tools for wheat breeding and dissecting the function of DELLA proteins. *Journal of Experimental Botany*, *68*(3), 353-358. https://doi.org/10.1093/jxb/erw509

Trethowan, R. M., Singh, R. P., Huerta-Espino, J., Crossa, J., & van Ginkel, M. (2001). Coleoptile length variation of near-isogenic *Rht* lines of modern CIMMYT bread and durum wheats. *Field Crops Research*, *70*(3), 167-176. https://doi.org/10.1016/S0378-4290(00)00153-2

Tsai, H., Howell, T., Nitcher, R., Missirian, V., Watson, B., Ngo, K. J.,…Comai, L. (2011). Discovery of Rare Mutations in Populations: TILLING by Sequencing. *Plant Physiology*, *156*(3), 1257-1268. https://doi.org/10.1104/pp.110.169748

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M.,…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

USDA. (2024). *Crops and crop products: Grains and oilseeds.* In *Small grains annual summary* (ID: 5t34sj573). United States

CHAPTER FIVE

A *TEOSINTE BRANCHED-B1* NULL MUTATION INCREASES DURUM WHEAT TILLERING, INCREASING GRAIN YIELD IN CERTAIN ENVIRONMENTS

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Abstract

Wheat (*Triticum* spp.) is a hardy, drought-tolerant crop well suited to harsh environments like the Northern Great Plains. In regions with higher rainfall or irrigation, a densely planted, high-biomass crop ideotype may be preferable. However, in moisture-stressed climates with variable weather, phenotypic plasticity is crucial for maintaining high yield potential. Drought-tolerant genotypes with phenotypic plasticity can provide a harvestable crop in poor years while maximizing yields in favorable conditions. Greater tillering plasticity allows plants to capitalize on timely rainfall, ultimately increasing grain yield. *Teosinte Branched-1 (TB1)* is a transcription factor that regulates axillary meristem outgrowth in wheat. This study examines its effects on tillering, mature inflorescence morphology, and their impact on grain yield in durum wheat *(Triticum turgidum* L. subsp. *durum)*. Reducing *TB1* function through nonsense mutations in one homoeolog can enhance tillering plasticity, boosting yield under favorable conditions. *TB1* variants were analyzed in near isogenic line populations across three years and five Montana environments. Lines with mutations in both *TB1* homoeologs had 20 % more productive tillers, but reduced grain yields in some environments due to reduced spike size. Genotypes containing only the *tb-B1-W341** nonsense mutation allele had grain yield increase of up to 20 % in environments with optimal mid-season rainfall and did not yield significantly lower than wildtype genotypes in any other environment. Integrating a *TB-B1* nonsense allele into durum wheat breeding programs will increase tillering plasticity and yield potential.

Introduction

Wheat (*Triticum* spp.) is a major component of the human diet around the world. It is a cool season crop with both spring and winter growth habits and can produce harvestable grain yields under drought. These traits make it a top choice for production in harsh climates with primarily rainfed (non-irrigated) production, such as the Northern Great Plains region of the United States. Durum wheat *(Triticum turgidum* L. subsp. *durum)* is a tetraploid species of wheat primarily grown and used to produce semolina used in pasta production. The Northern Great Plains is the primary growing region for durum wheat in North America, with production in Montana, North Dakota, Saskatchewan, and Alberta totaling 7.7 million metric tons in 2024, comprising almost one quarter of durum wheat production worldwide (USDA, 2024; AAFC, 2024; Peterson, 2024). This study was conducted in tetraploid durum wheat to support durum breeding programs in the Northern Great Plains. Additionally, studies in tetraploid wheat (AABB) provide a strong foundation for research in hexaploid wheat (AABBDD) since absence of the D genome simplifies experimental design, particularly when introducing *TB1* mutations in each homoeolog. High levels of synteny between the A and B genomes of hexaploid and tetraploid wheat mean most conclusions from tetraploid experiments can be translated to hexaploid wheat (El Baidouri et al., 2017).

Teosinte Branched-1 (*TB1*) regulates plant axillary meristem outgrowth and controls a key genetic difference between modern maize (*Zea mays* L. subsp. *mays*) and its ancestral species, teosinte *(Zea mays* L. subsp. *parviglumis)* (Doebley et al., 1995). The *TB1* allele found in Maize is expressed at twice the level of the allele found in teosinte. This higher expression is a result of a transposon insertion in the promoter region (Studer et al., 2011). This greater

expression results in a single stalk or branch in maize, while teosinte has multiple stalks or branches (Doebley et al., 1997; Doebley et al., 1995). *TB1* orthologues have been identified in barley (*Hordeum vulgare* L.) referred to as either *HvTB1*, *Int-C* (*INTERMEDIUM-C*), or *Vrs-5* (*Six-Rowed-Spike-5*) (Alqudah et al., 2016; Ramsay et al., 2011; Shang et al., 2020; Zwirek et al., 2019); rice (*Oryza sativa* L.) referred to as *OsTB1* (Takeda et al., 2003); and *Arabidopsis thaliana* (L.), referred to as *BRC1 (Branched-1)* (Aguilar-Martinez et al., 2007). The *TB1* orthologue in wheat species, sometimes referred to as *TaTB1*, has been studied much less thoroughly than the maize *TB1* gene. Variation in *TB1* expression in wheat has similar impacts as in maize, with expression negatively correlated with tillers (Lewis et al., 2008, Dixon et al., 2018). Dixon et al. (2018) demonstrated that increased *TB1* dosage increases paired spikelets while also decreasing tillers. Decrease in *TB1* function is known to increase tiller number and can also affect other morphological traits (Dixon et al., 2020; Volkman et al., 2022).

Dixon et al. (2018) described two naturally occurring *TB1* alleles, one on the B genome referred to as *TB-B1b*, and one on the D genome, referred to as *TB-D1b*. The *TB-B1b* allele contains three missense changes within the predicted sequence while *TB-D1b* contains only one missense change relative to reference *TB1* alleles, *TB-B1a* and *TB-D1a*, respectively. Both *TB-B1b* and *TB-D1b* are present among Montana grown spring wheat varieties (Volkman et al., 2022). Dixon et al. (2018) demonstrated that both alleles decreased binding of TB1 to FT (FLOWERING TIME) in yeast two-hybrid assays. No *TB1* allelic variation has been reported in durum wheat. Severe *TB1* mutations would not exist naturally since this gene is an important part of the shade response pathway, enabling plants to compete with one another in nature. When plants experience shading, *TB1* expression is promoted, decreasing tillering (Wang et al., 2022;

Whipple et al., 2011). Any reduced ability to respond to shading in a grassland would be selected against and would not survive evolutionary pressure. While there are many studies that focus on either upregulation or full knockouts of *TB1* and its homologues, both of which can have negative effects on crop yields, few studies have looked at partial knockouts or reduced function of *TB1* to increase branching and yield of crops in which a more highly branched phenotype may be beneficial.

Maize, which is traditionally grown in warmer wetter regions of North America, has undergone directional selection for a taller, single stalked phenotype, facilitated in large part by increased *TB1* expression levels. Unbranched maize with erect leaves yields well planted at high densities in high resource environments due to its ability to capture more sunlight (Jafari et al., 2024; Mock & Pearce, 1975). Modern wheat is inherently drought, heat, and cold tolerant compared to crops such as corn and soybeans (*Glycine max* L.), which require more water and longer growing seasons. Because of the climates in which wheat is grown, yields are most commonly limited by water availability and not by sunlight (Kukal & Irmak, 2018; Mueller et al., 2015; Thomashow, 1998). Donald (1968) presented a model for the ideal wheat ideotype following the logic of modern maize breeding: an unbranched, single stalked plant grown at high densities in high resource environments. This ideotype describes plants that are communal and not competitive, inducing minimal plant to plant interference enabling maximum yields in a monoculture (Donald, 1981). This study approaches the communal crop ideotype in a different way. By reducing plant shade response through reduced *TB1* function, plants grown in a monoculture are partially shade blind and more free tillering. This approach decreases competitive plant interactions by decreasing a plant's ability to detect its neighbors, instead of by

reducing physical interference. This would reduce the impact of shading and sunlight on tillering and would put more emphasis on belowground resources as determinants for tillering potential.

Later studies confirm that wheat lines with less tillering do well in high resource environments at high planting densities (Duggan et al., 2005; Sedgley, 1991). However, these lower tillering lines do not confer a yield advantage in drought conditions, and studies also note that increased tillering potential may allow for increased yields at lower planting densities and in environments with erratic rainfall or low fertility (Tokatlidis, 2014; Whan et al., 1988; Yunusa & Sedgley, 1992). Additionally, increased tillering through introgression of the *QTn.mst-6B* QTL increases grain yield in Montana environments with higher moisture or decreased planting density (Jones et al., 2021; Naruoka et al., 2011; Nasseer et al., 2016). When developing drought tolerant wheat, breeders often focus on traits like increased heat tolerance at flowering to decrease spike sterility, the stay green trait to lengthen grain fill periods, and assimilate remobilization efficiency to improve grain fill during senescence (Bazargani et al., 2011; Cook et al., 2021; Dong et al., 2017; Liu et al., 2020; Semenov & Stratonovitch, 2013; Senapati et al., 2019). These traits can reliably improve grain yields in drought prone environments and are not susceptible to genotype by environment interactions. Increasing tillering plasticity would likely improve grain yield primarily in years with timely mid-season rainfall, making this trait more prone to environmental interactions. Although difficult to study, traits that enable adaption to unpredictable weather patterns are valuable since erratic seasonal rainfall is a characteristic of drought prone environments.

Following this logic, *TB1* was chosen as a target for modification in wheat. In direct opposition to the unbranched phenotype of the ideal maize plant grown in a high resource

environment, *TB1* expression would be reduced to create phenotypes with increased tillering plasticity that can take advantage of limited water resources in the harsh growing conditions of the Northern Great Plains. In this study, ethyl methanesulfonate (EMS) induced stop mutations in *TB-A1* and *TB-B1* homoeologs are tested separately and in combination in a Montana adapted durum wheat background. These lines were grown across 11 different location-years in yield trials and were also planted in variable rate seeding experiments across three location-years to determine how and when *TB1* mutations can benefit grain yield. Tillering, spike morphology, and other parameters were measured to determine what yield components are affected by *TB1* mutations.

Materials and Methods

Creation of Isogenic Lines

Two EMS derived mutant lines were selected from the 'Kronos' TILLING database, Kronos994 and Kronos562. These lines contain the nonsense mutant alleles *tb-A1-W339** and *tb-B1-W341** respectively (Krasileva et al., 2017). These lines were crossed together to make a double *TB1* mutant line. This line was crossed with experimental semidwarf durum line 'MT112219' and then backcrossed three times, before crossing twice with the variety 'MT Raska'. MT Raska (PI 703026) is a recently released semidwarf variety that is higher yielding than MT112219 (Hogg et al., 2025). Resulting BC_1F_1 seed was planted and seedlings were genotyped. The healthiest and highest yielding BC_1F_1 plant heterozygous for both *TB-A1* and *TB-B1* was selected for advancement. From genotyped BC_1F_2 seedlings, four plants from each of the four combinations of fixed *TB1* allele groups were selected (full wildtype, *tb-A1*-W339* single mutant, *tb-B1*-W341* single mutant, and *tb-A1*-W339* *tb-B1*-W341* double mutant) for a

total of 16 BC_1F_2 derived near isogenic lines (NIL). Seed from these plants was increased in the greenhouse in 30 cm wide pots, with two pots of five seeds planted per each NIL. Resulting $BC_1F_{2:3}$ seed was used to plant field experiments in Bozeman, MT in 2022. $BC_1F_{2:4}$ seed harvested from the Bozeman 2022 irrigated field trial was used to plant all trials in 2023 and 2024.

Genotyping

At each backcrossing and line selection step, plants were genotyped for the presence of both *tb-A1-W339** and *tb-B1-W341** using standard PCR and sanger sequencing based on the protocol described in Volkman et al. (2022). PCR conditions were modified slightly, reducing the concentrations of primers to reduce off target amplification. For *TB-A1,* reagent levels per single 25 µl reaction were 14.27 µl of ultrapure nuclease-free water, 5 µl of 5x Green GoTaq® Flexi Buffer (Promega, Madison, WI), 2 µl of $MgCl_2$, 2 µl of dNTP at a concentration of 2 mM, 0.2 µl of 20 mM forward primer, 0.2 µl of 20 mM reverse primer, and 0.13 µl of taq G2 polymerase. Reagent levels for amplification of *TB-B1* were similar but with the concentration of forward and reverse primers set at 0.4 µl per 25 µl reaction. Primers used for amplification and sequencing are published in (Volkman et al., 2022). PCR conditions for both genes were 40 cycles of 30 seconds of denaturation at 96 °C, 30 seconds of annealing at 65 °C, and one minute of extension at 72 °C. Sequence was analyzed using SeqMan Pro® Version 17 (DNASTAR, Madison, WI).

Allele specific qPCR PACE® genotyping markers (3CR Bioscience, UK) were later developed to genotype for *tb-A1-W339** and *tb-B1-W341** alleles (Table 1). Developed primer sequences are shown in Table 5.1. Each 10 µl reaction contained 1.2 µl of genomic DNA at a

concentration of approximately 2 µg/µl, 3.66 µl of ultrapure nuclease-free water, 5 µl of PACE® master mix, and 0.14 µl of primer stock mix. Batches of 100 µl of stock primer mix were prepared by combining 100 µm primer stocks at the following ratio: 12 µl of hex tagged primer, 12 µl of fam tagged primer, 30 µl of common primer, and 46 µl of 10 mM Tris buffer with a pH of 8.5. PCR conditions involved a touchdown PCR protocol with an initial denaturation at 94 °C for 15 min followed by cycles of denaturation at 94 °C for 20 sec with a combined annealing and extension of 65 °C for 1 min that decreased by 0.8 °C each cycle for 10 cycles. This touchdown sequence was followed by 29 cycles of 94 °C denaturation for 20 sec, 57 °C of combined annealing and extension for 1 min, and a plate read step at 30 °C for 30 sec.

Table 5.1. Description and sequences of primers developed for use in allele specific qPCR PACE® marker assays to genotype for mutant alleles *tb-A1*-W339* and *tb-B1*-W341*

Allele	Primer Type	Primer Name	Primer Sequence
tb-A1-W339*	Wildtype allele specific	TBA1 348 Hex Rev2	5' HEX-CCACTCCAGCGAGCTCCC 3'
	Mutant allele specific	TBA1 348 Fam Rev2	5' FAM-CCACTCCAGCGAGCTCCT 3'
	Common	TB1A For7	5' ATGCCGAGCGAAGCTATCG 3'
tb-B1-W341*	Wildtype allele specific	TBAB1 348 HEX for	5' HEX-TATCAGCTGGAGCAGCAATGG 3'
	Mutant allele specific	TBAB1 348 FAM for	5' FAM- TATCAGCTGGAGCAGCAATGA 3'
	Common	TBB1 Rev	5' GGGCTGGGAGTTGGGAAA 3'

Field Experiments

Experiments examining MT Raska background *TB1* NIL were grown in 11 experiments across four locations and three years (Table 5.2). Each experiment was set up as a randomized complete block design, with each replicate including all 16 NIL and two MT Raska parent checks. Experiments were planted near Bozeman, MT at the Arthur H Post Agronomy Farm in

2022, 2023, and 2024 in two separate yet adjacent experiments with two replicates each. In 2022, one of these experiments was irrigated with hand line sprinklers to add approximately 15 cm of irrigation water in 5 cm increments on 21 and 26 June and 2 July. In 2023, this location received above average growing season rainfall (Table 5.3), and the two experimental blocks were treated as one rainfed experiment with four replicates. In 2024, one of these experiments was irrigated to add approximately 5 cm of irrigation water on 7 July. Similar experiments were planted in 2023 and in 2024 near Havre, MT at the Montana State University Northern Agricultural Research Center; in Sidney, MT at the Eastern Agricultural Research Center; and in Moccasin, MT at the Central Agricultural Research Center. In 2023, two replicates were planted at each research center, and in 2024, three replicates were planted at each research center. See Table 5.2 for all planting dates, harvest dates, seeding rates, and row spacing for each trial. See Table 5.3 for average monthly temperatures and total water received (including irrigation) for each experiment. All trials received proper amounts of nitrogen and phosphorus fertilizer and were treated with appropriate herbicides and pesticides according to field conditions. Seed planted in all 2023 and 2024 trials was treated with CruiserMaxx® Vibrance® (Syngenta U.S., Greensboro, NC).

Table 5.2. Different field management details at different locations and years in which experiments in this study where planted. [a]In Bozeman seeding rate experiments, plots were planted at high seeding rate of 391 seeds m^{-2}, a standard seeding rate of 196 seeds m^{-2}, a low rate of 98 seeds m^{-2}, and a very low rate of 42 seeds m^{-2}.

Year	Location	Water Treatment	Reps	Rows/ Plot	Plot Length	Row Spacing	Plot Area	Seeding Rate	Plant Date	Harvest Date
			——no——		m	cm	m^2	seeds m^{-2}	——date——	
2022	Bozeman	Rainfed	2	2	3.0	30	1.9	196	6 May	12 Sep
2022	Bozeman	Irrigated	2	2	3.0	30	1.9	196	6 May	12 Sep
2023	Bozeman	Rainfed	4	2	3.0	30	1.9	multiple[a]	5 May	15 Sep
2023	Havre	Rainfed	2	3	5.5	30	4.5	233	29 Apr	4 Aug
2023	Moccasin	Rainfed	2	5	3.0	30	4.6	224	3 May	15 Aug
2023	Sidney	Rainfed	2	7	3.0	18	4.6	231	18 Apr	8 Aug
2024	Bozeman	Rainfed	2	2	3.0	30	1.9	multiple[a]	24 Apr	27 Aug
2024	Bozeman	Irrigated	2	2	3.0	30	1.9	multiple[a]	16 Apr	23 Aug
2024	Havre	Rainfed	3	3	5.5	30	4.5	233	24 Apr	27 Aug
2024	Moccasin	Rainfed	3	5	3.0	30	4.6	224	23 Apr	20 Aug
2024	Sidney	Rainfed	3	7	3.0	18	4.6	231	15 Apr	1 Aug

Table 5.3. Monthly precipitation and temperature data for each location and year in which experiments were planted. [a]Last frost recorded on last calendar day on which there was an observed air temperature of < 0 C°. [b]No data recorded this month.

				—— Water Received——						—— Average Temperature ——					
Year	**Location**	**Water Treatment**	**Last Frost[a]**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Season Total**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Season Avg.**
			date	cm						C°					
2022	Bozeman	Rainfed	31 May	4.1	11.0	6.0	1.4	1.5	24.0	2.9	9.1	15.4	20.6	21.7	13.9
2022	Bozeman	Irrigated	31 May	4.1	11.0	16.0	6.4	1.5	29.0	2.9	9.1	15.4	20.6	21.7	13.9
2023	Bozeman	Rainfed	22 Jun	1.9	1.9	12.9	2.5	4.7	23.9	4.2	13.3	14.4	19.7	19.4	14.2
2023	Havre	Rainfed	28 Apr	1.4	7.4	5.7	1.6	2.5	18.6	4.7	14.9	17.3	21.1	21.1	15.8
2023	Moccasin	Rainfed	10 May	6.6	7.7	13.3	0.5	1.3	29.3	3.9	13.2	14.7	19.3	19.3	14.1
2023	Sidney	Rainfed	30 Apr	1.0	nd[b]	4.5	2.9	7.4	15.9	9.0	19.0	23.2	23.9	24.9	20.0
2024	Bozeman	Rainfed	31 May	3.6	8.9	4.1	1.1	2.6	20.3	7.4	9.5	16.2	20.4	18.8	14.5
2024	Bozeman	Irrigated	31 May	3.6	8.9	4.1	6.1	2.6	25.3	7.4	9.5	16.2	20.4	18.8	14.5
2024	Havre	Rainfed	4 May	2.2	11.0	6.3	1.8	3.7	25.0	7.3	11.0	15.3	21.8	20.1	15.1
2024	Moccasin	Rainfed	1 Jun	6.5	9.8	7.1	1.3	2.8	27.5	5.8	8.2	13.9	20.0	18.9	13.4
2024	Sidney	Rainfed	21 Apr	0.9	8.8	4.6	1.5	1.3	17.2	12.6	16.2	19.9	25.4	24.4	19.7

In 2023 and in 2024, variable seeding rate trials were added to experiments planted in Bozeman, MT. Standard seeding rate in all Bozeman, MT experiments was equivalent to approximately 196 seeds m^{-2}. Three additional seeding rates were applied, including a double of standard 'high' seeding rate of 391 seeds m^{-2}, a half of standard 'low' rate of 98 seeds m^{-2}, and a slightly less than quarter of standard 'very low' rate of 42 seeds m^{-2}. Seeding rate experiments were set up as randomized split plot designs, with seeding rate as the main plot and *TB1* NIL as the sub plot. This design eliminated potential interactions from neighboring plots of differing densities, and was designed such that data from standard seeding rate plots could be analyzed together with other standard seeding rate trials.

Phenotyping

In all 11 standard density trials, mature tiller number and productive spike number was recorded from a representative 30 cm section of row. Counts were performed at physiological maturity. Physiological maturity was determined when at least half of peduncles in a plot had turned brown. Equivalent tiller number per square meter was calculated based on these measurements and according to the row spacing of each trial. Plant height was also measured at maturity, determined by the distance from soil surface to the top of representative primary spikes, not including awns. Before harvest when grain had fully matured (Zadoks 92), five spikes from primary tillers were randomly selected from each plot for analysis (Zadoks et al., 1974). Spikes were left to dry fully, number of spikelets per spike was recorded, and all five spikes from each plot were threshed together by hand. Grain from the five spikes was counted and weighed. From this data, average spikelets per spike, average grain weight per primary spike, average grain number per primary spike, and average grain number per spikelet was calculated. Spikes

were not collected from the rainfed Moccasin 2023 environment. In 2022 in Bozeman, plots were harvested using a single row binder made by Mitsubishi (Mitsubishi Mahindra Agricultural Co., Ltd., Higashiizumo, Shimane, Japan), and bundles were threshed using a Vogel threshing machine. In all other years and locations, plots were harvested using a small plot combine. Grain yield was recorded as the weight of uncleaned harvested grain from each plot, and equivalent kg ha^{-1} was calculated. Grain protein content and individual seed weight were measured for all experiments in all locations, including seeding rate trials. Grain protein content of cleaned grain was measured by near-infrared transmittance using a Foss Infratec 1241 Grain Analyzer (Foss North America, Silver Springs, MD) and seed size was determined by weighing a random sample of 200 seeds from cleaned subsamples.

In standard seeding rate experiments planted in Bozeman in 2022, 2023, and 2024, tillers were counted within the same section of row at three different timepoints across the growing season. At Zadoks stage 25 (tillering/vegetative stage) a representative 30 cm section of row without significant gaps was selected and marked with stakes (Zadoks et al., 1974). The total number of tillers between these stakes was first counted at Zadoks stage 31 (first node). The second tiller count was performed at Zadoks stage 59 (heading). The third tiller count and productive spike count was performed at physiological maturity, as in all other locations. See Table 5.4 for dates of tiller counts at each growth stage for each year. Additionally, in Bozeman standard density experiments, three flag leaves from randomly selected primary tillers were measured in width at their widest point, and in length from the edge of the ligule to the tip of the leaf, after heading but before physiological maturity. Primary tillers were determined to be tillers that appeared taller and more mature. Heading date and maturity date was recorded for all

experiments planted in Bozeman, including all variable seeding rate experiments. Heading date was determined by the Julian date on which 50 % of spikes in the plot had fully emerged from the sheath.

Table 5.4. Timepoints of tiller counts at different growth stages in Bozeman experiments

Environment		**Tiller Count Date**		
Year	**Treatment**	**Jointing**	**Heading**	**Maturity**
2022	Rainfed	25 June	11 July	12 August
2022	Irrigated	25 June	11 July	15 August
2023	Rainfed	15 June	07 July	18 August
2024	Rainfed	13 June	01 July	26 July
2024	Irrigated	16 June	03 July	05 August

Statistical Analysis

Mixed model analysis of variance using the lme4 package (Bates et al. 2015) in R (R Foundation for Statistical Computing. Version 4.0.5. Vienna, Austria) was used to analyze all data from this study. Mean response variable values of the four NIL representing each genotype group within each replication were calculated. This resulted in one mean value for each genotype group within each technical replication. These mean values were used to analyze data for all experiments in this study. Each location, year, and water treatment combination was treated as a unique environment. For response variables measured in standard planting density experiments, *TB1* genotype, environment, and their interaction were considered fixed effects, while block within environment was considered a random effect. Due to a highly significant genotype-environment interaction (<0.0001), a type III analysis of variance test was used to determine significance between these effects on grain yield. For all other response variables, there was little

to no significant genotype-environment interaction, so a type II analysis of variance test was used. To analyze grain yield data from the seeding rate experiments conducted in Bozeman in 2023 and 2024, a mixed linear model that included genotype, environment, density, and their interactions were all considered as fixed effects. Block within each environment and density was considered a random effect, with random slopes for density at the replicate level within each environment-year-location, allowing for different relationships between density and the response variable across these levels. Type II analysis of variance was used to analyze grain yield variation across planting densities in this experiment. For all analyses, least significant difference values were calculated to indicate variance, and to determine significant differences following a significant genotype effect. Pairwise comparisons between genotypes were also performed for select traits.

Results

Overall Results

A stepwise increase in tiller number was observed on average across all environments, with single *tb-B1*-W341* mutant lines and double mutant lines (*tb-A1*-W339* and *tb-B1*-W341*) producing significantly more tillers and productive spikes than the wildtype (Table 5.5). Single *tb-B1*-W341* mutant lines produced more tillers and productive spikes than *tb-A1*-W339* single mutant lines, although this difference was not statistically significant. Although the double mutant lines had more productive spikes, these spikes were smaller, producing lower grain yields per spike. This resulted in the double mutant genotype lines producing lower grain yield than wildtype lines (Table 5.5). Both single mutant genotypes produced slightly smaller spikes than wildtype. However, these decreases in spike size were overshadowed by increases in productive

spikes such that average grain yield was increased in single mutant genotypes relative to wildtype, although this difference was not statistically significant overall (Table 5.5). Spike size differences between genotypes were primarily due to significant differences in seeds per spikelet and in spikelets per spike, reducing overall seed number per spike. On average, there were no significant differences in single grain weight between genotypes, although there was a significant genotype by environment interaction for this parameter (Table 5.5). Double mutant genotypes had significantly larger single grain weights in the rainfed Bozeman 2023 environment, but significantly smaller single grain weights in Moccasin 2023 and 2024 environments (Table C5.6). See Table 5.5 for mean values across all environments and Supplementary Tables S1-S11 for mean values within each environment.

Table 5.5. Mean agronomic trait values of MT Raska background NIL genotype groups measured across multiple environments. The single mutant *tb-B1*-W341* genotype group yielded the highest on average, maintaining normal single grain weight and high grain protein content.

Genotype		**Grain Yield**	**Mature Tillers**	**Productive Spikes**	**Height**	**Spikelets/ Spike**	**Single Grain Weight**	**Yield/ Spike**	**Seeds/ Spikelet**	**Grain Protein Content**
TB-A1 Allele	*TB-B1* Allele	kg ha^{-1}	tillers m^{-2}	tillers m^{-2}	cm	no	mg	g	no	g kg^{-1}
TB-A1a	*TB-B1a*	4104	547.9	493.0	69.9	14.8	33.6	1.51	2.85	140
tb-A1-W339*	*TB-B1a*	4129	559.7	505.9	67.1	14.9	33.5	1.44	2.71	143
TB-A1a	*tb-B1*-W341*	4154	580.2	518.8	68.4	14.3	33.6	1.43	2.79	141
tb-A1-W339*	*tb-B1*-W341*	4033	603.9	537.1	67.1	14.2	33.3	1.34	2.69	144
	$LSD_{0.05}$	**94.7**	**21.6**	**19.3**	**0.51**	**0.16**	0.50	**0.033**	**0.054**	**1.6**
	No. Tests	11	11	11	11	10	11	10	10	11
P value	Genotype	0.002	<0.0001	<0.0001	<0.0001	<0.0001	0.504	<0.0001	<0.0001	<0.0001
	Environment	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	GXE	<0.0001	0.150	0.200	0.660	0.729	0.010	0.068	0.142	0.033

In 2022, both single mutant genotypes produced significantly higher grain yield than wildtype. In the 2022 Bozeman rainfed environment, the single mutant *tb-B1*-W341* lines yielded 20.1 % more grain than the wildtype lines ($p = 0.009$, independent-sample t-test) and in the 2022 Bozeman irrigated environment they yielded 15.4 % more ($p < 0.0001$, independent-sample t-test) (Table C5.1). In the 2022 Bozeman rainfed experiment, *tb-B1*-W341* single mutant lines had 6 % more productive spikes than the wildtype, and in the irrigated environment they had 17 % more productive spikes (Table C5.3). These lines had slightly reduced spike size, but seed weight increased slightly, and flag leaf size was increased in the rainfed environment (Tables S6, S7, S10). The *tb-B1*-W341* single mutant lines did not yield significantly more or less than the wildtype in other environments in standard planting density experiments (Table C5.1).

Plant height differed significantly between genotypes. The *tb-B1*-W341* single mutant lines were 1.5 cm shorter than wildtype lines ($p < 0.0001$, independent-sample t-test). The *tb-A1*-W339* single mutant lines and *tb-A1*-W339*/*tb-B1*-W341* double mutant lines were 2.8 cm shorter than wildtype lines ($p <0.0001$, independent-sample t-test) (Table 5.5). Although small, this height difference was consistent across all environments (Table C5.4). All genotypes shared the same heading dates and maturity dates within tested environments (data not shown). Across all environments, all three mutant genotype classes produced grain with higher grain protein content than the wildtype genotype. This increase was statistically significant in *tb-A1*-W339* single mutant lines ($p = 0.0002$, independent-sample t-test) and *tb-A1*-W339*/*tb-B1*-W341* double mutant lines ($p <0.0001$, independent-sample t-test) (Table 5.5). Grain protein content differences generally trended in the same directions but at different magnitudes resulting in a

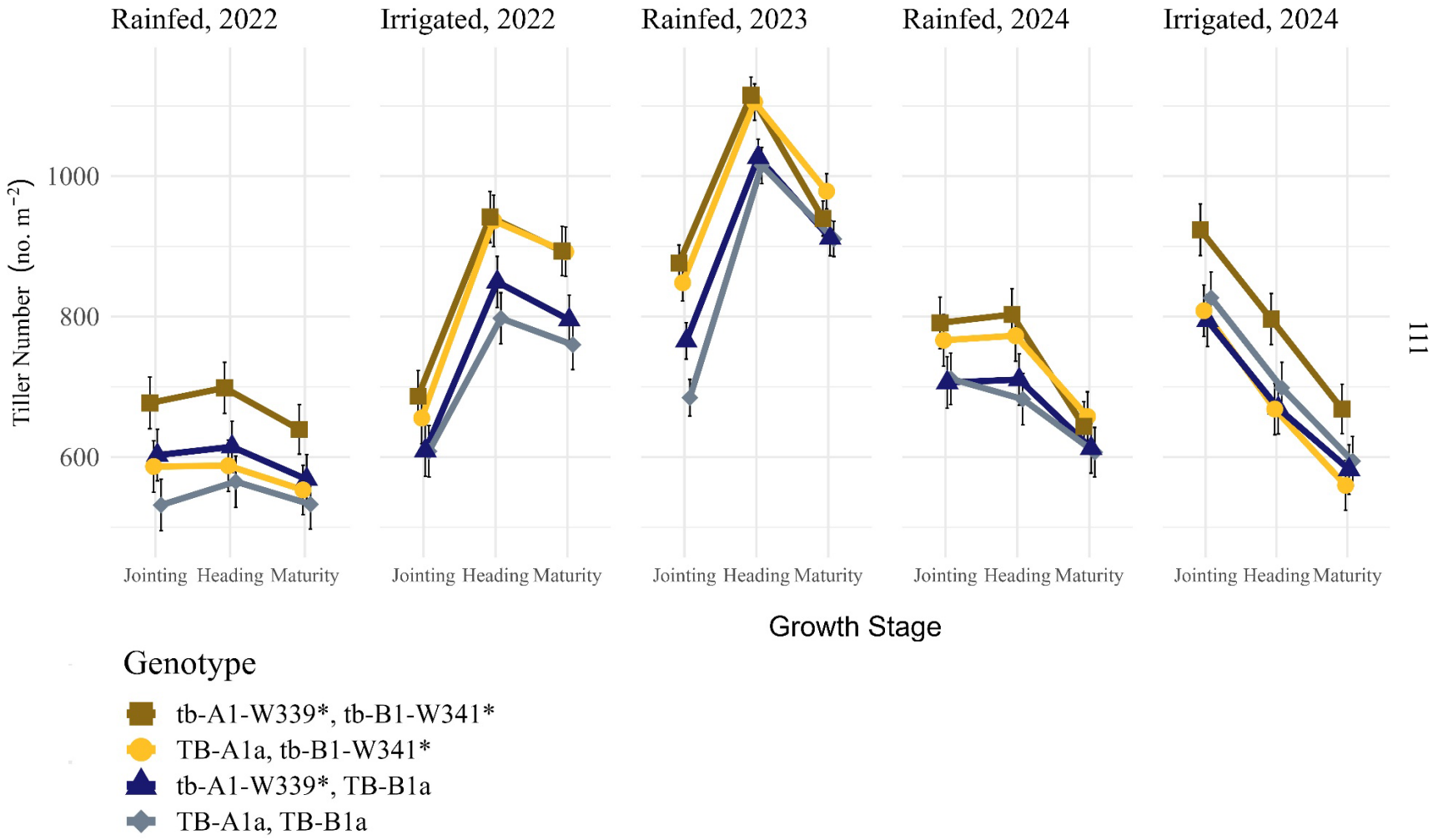
significant genotype-environment interaction ($p = 0.03$) (Table C5.9). There were no significant differences in leaf length or leaf width, although the double mutant genotypes trended towards having reduced flag leaf length of approximately 1.0 cm depending on the environment (Tables S10, S11).

Early and Mid-Season Tiller Count Results

Tiller counts performed at three timepoints across the season in Bozeman, MT revealed differences between genotypes, and overall differences between years and environments (Figure 1). For all genotypes, an overall increase in tiller number between jointing and heading, followed by an overall decrease in tillers between heading and maturity was observed in all environments except for the 2024 irrigated environment, in which an overall decrease in tiller number was observed after jointing. The *tb-B1*-W341* single mutant lines generally produced more tillers than wildtype, although this difference was significantly higher only in the 2023 rainfed environment during jointing. The *tb-A1*-W339* and *tb-B1*-W341* double mutant lines generally produced many more tillers than wildtype lines, although this difference not statistically significant at jointing in the 2022 irrigated environment, or at maturity in 2023 and 2024 environments. The single *tb-B1*-W341* mutant genotype displayed the most variability between growth stages and environments relative to other genotypes. In the 2022 rainfed environment, this genotype produced slightly more tillers than wildtype, but less tillers than other mutant genotypes. In the 2022 irrigated environment, this genotype started out with slightly more tillers than the wildtype genotype at jointing, but tiller numbers increased to similar levels as the double mutant genotype lines at heading and maturity. In the 2023 and 2024 rainfed environments, this genotype produced similar tiller numbers to the double mutant lines, ending the growing season

at maturity with more tillers than any other genotype, although differences were not statistically significant. Finally, in the irrigated 2024 environment, the single *tb-B1*-W341* mutant genotype trended towards producing less tillers than wildtype across all three timepoints (Figure 1).

Figure 5.1. Tiller number differences between *TB1* genotypes across growing season in five Bozeman environments. Average tiller numbers of the single mutant *tb-B1*-W341* genotype group relative to other genotype groups highlight the high tillering plasticity of lines containing this allele.



Seeding Rate Trial Results

As noted previously, standard seeding rate experiments (196 seeds seeds m^{-2}) in Bozeman in 2023 and 2024 did not show any significant grain yield differences between genotypes. However, at a low seeding rate (98 seeds m^{-2}) in 2023, the *tb-B1*-W341* single mutant genotype yielded 13.0 % more than the wildtype lines ($p = 0.0012$, independent-sample t-test) (Supplementary Figure S1). In fact, this genotype at a low seeding rate yielded slightly more than the wildtype genotype at a standard seeding rate, although this difference was not statistically significant. No significant grain yield differences between genotypes were detected across other tested densities and environments. Similar to all standard seeding rate trials, all genotypes within each variable seeding rate shared the same heading and maturity dates. Lines planted at low and very low seeding rates headed and matured one to two days later than lines planted at standard and high seeding rates (data not shown).

Discussion

Tillering Plasticity and Grain Yield

Results show that introducing mutant alleles of *TB-A1* and *TB-B1* into durum wheat causes increased tillering across environments, although the overall number of tillers present at maturity is certainly constrained by environmental factors such as water availability. Although tillering of these double mutant genotypes is much less constrained than wildtype, extra tillers wither away later in the growing season without adequate moisture availability. The *tb-B1*-W341* single mutant lines showed increased tillering plasticity between environments. In environments with lower moisture, like the 2022 rainfed Bozeman environment, these lines tillered at similar levels to the wildtype (Figure 1). In environments with more early growing

season water availability, such as the 2022 irrigated Bozeman and 2023 rainfed Moccasin environments, these single mutant lines tillered similarly to double mutant genotypes. The single *tb-B1*-W341* mutant lines yielded significantly more grain than the wildtype lines in Bozeman in 2022. This is likely in part because these plots were planted later than average, and the single *tb-B1*-W341* mutant lines' increased tillering plasticity allowed for rapid growth and tillering in the month of May which experienced above average rainfall (Table 5.3). Although there was similar early season rainfall experienced in the Havre 2024 experiment, these plots were planted earlier at a higher seeding rate, and experienced hotter temperatures in July, all factors that may have contributed to no realized difference in grain yield (Table 5.2 and Table 5.3). Overall, increased tillering plasticity resulting from the *tb-B1*-W341* single mutant genotype enables the crop to take advantage of mid-season moisture and translate it to increased productive tiller number and grain yield if conditions are right. If later season conditions do not allow for adequate grain fill, tiller increases are not so drastic such that it decreases yield compared to wildtype. Likewise, if there is not above average early season rainfall, single *tb-B1*-W341* mutant lines do not tiller significantly more, also resulting in similar yields compared to wildtype (Table C5.1, Table 5.3).

Since sterile tillers are known to reduce yield potential, it was important to measure total tiller number and productive tiller number to determine if *TB1* mutations increase this undesirable trait (Richards, 1988). On average, wildtype and single mutant genotypes produced productive tillers at an identical rate (90% of total tillers). Double mutant genotypes produced productive tillers at a slightly lower rate (89 %) although this decrease was not significant. Increased production of early tillers in *TB1* mutants keep this rate consistent since early tillers

are much more likely to produce fertile spikes than later tertiary tillers. (Clements et al., 1974; Ishag & Taha, 1974)

Early and mid-season tiller counts tell us more about the tillering plasticity of *TB1* mutant lines. In the 2022 Bozeman experiments, rainfed and irrigated tiller counts at jointing were performed on the same day. Water had been applied to the irrigated environment four days prior to these counts. This irrigation event did not cause an increase tillers in double *TB1* mutant genotypes relative to the non-irrigated experiment, but it did increase the number of tillers produced in other genotypes, reducing differences in tiller number between genotypes at jointing. In the 2023 Bozeman environment, there was very little rainfall in May, but higher than average rainfall in June, causing much higher overall tiller counts. In the 2024 Bozeman environments, there were more tillers at jointing in the irrigated experiment than in the rainfed experiment, even though no irrigation water was applied until after jointing. This is likely due to an earlier planting date for the rainfed as well as spatial variability between these two experiments. In rainfed environments in 2023 and in 2024, single *tb-B1*-W341* mutant lines ended the season with more tillers than other genotypes, presumably because there was not adequate moisture for double mutant genotype plants to sustain the high tiller numbers they produced early in the season (Figure 1). These differences across environments and years highlight the tillering plasticity and environmental adaptability of single *tb-B1*-W341* mutant lines relative to other genotypes.

The effect of this tillering plasticity on yield can be exacerbated when seeding rate is decreased. In 2023, *tb-B1*-W341* single mutant lines produced high grain yields in the low seeding rate environment because of their increased ability to tiller given increased space and resources, despite experiencing shade from neighbors. Genotypes planted at the very low seeding

rate experienced very little shading from neighboring plants, potentially downregulating *TB1* across all genotypes, increasing tillering and productivity regardless of genotype. Although no differences were seen across seeding rate experiments in 2024, 2023 data suggests that plants with reduced *TB1* function can partially ignore shading cues from neighboring plants and more completely utilize available resources.

Expression levels of *TB1* Homoeologs

Differences in performance between the two single mutant genotypes could be explained by differences in expression levels between the *TB-A1* and *TB-B1* homoeologs, although this explanation was not further explored. According to Dixon et al. (2018), *TB-A1* in hexaploid wheat is hardly expressed relative to *TB-B1*. Conversely, according to data published in the wheat expression browser and in papers that performed quantitative reverse transcription PCR on *TB1* orthologues in wheat, *TB-A1* and *TB-B1* are expressed at similar levels (Borrill et al., 2016; Gonzalez-Grandio et al., 2017; Sigalas et al., 2024). *TB1* is a transcription factor and is relatively lowly expressed in general, making it difficult to detect in transcriptomics studies. Phenotypic data from this study shows that *TB-A1* knockout alleles confer an effect on tillering and other traits when compared to wildtype, but that single *TB-B1* knockouts confer a larger effect. This suggests a middle ground between conclusions of previous studies. Most likely, *TB-B1* is expressed at higher levels which confer a greater effect on phenotype relative to *TB-A1,* which is expressed at meaningful levels.

TB1 Affects Height

One finding in conflict with previous studies is the observed stepwise height decrease in *TB1* mutant lines (Table 5.5). This is not consistent with Dixon et al. (2020) which showed that

natural mutant alleles of *TB1* in hexaploid wheat increase plant height in greenhouse conditions. This difference could be a result of functional differences between EMS derived nonsense mutation alleles and natural mutant alleles, or it could be the result of a genotype by environment interaction based on whether plants were grown in the field or the greenhouse. In wheat species, *TB1* is closely linked to the Green Revolution gene *Reduced Height-1 (Rht-1)* on the group 4 chromosomes (Dixon et al., 2018). *Rht-1* affects many plant characteristics including plant height and tiller number (Brown et al., 2022; Jobson et al., 2018; Lanning et al., 2012). *TB1* also affects these traits, however transcriptome analysis of *Rht-1* overexpression lines has shown no differential expression of *TB1* homoeologs, indicating that there is no functional link between these two important genes (Xu et al., 2023).

Supporting Research

Results showing increased yields in *TB1* single mutant lines are indirectly supported by two studies linking reduced *TB1* expression or function to higher grain yields in stress-inducing environments. Zhou et al. (2022) found that introduction of the soybean transgene *GmTDN1* in wheat plants decreased *TB1* expression by about half. These lines exhibited increased drought and low nitrogen tolerance, and produced more productive tillers. Grain yields were significantly improved in rainfed, lowly irrigated, and low nitrogen conditions, while yield in well-irrigated, standard nitrogen environments was not statistically improved. Lowly irrigated treatments in Zhou et al. (2022) applied irrigation similarly to normally irrigated treatments in this study. It is important to note that hand line irrigated conditions in Bozeman, MT still represent a relatively stressed environment compared to experiments conducted in other regions. Similarly, Ishizaki et al. (2023) used CRISPR/Cas9 to create *OsTB1* missense mutations in rice, enhancing tillering

potential and increasing yields in low-phosphorus greenhouse trials, but not in nutrient-rich soils. Conducted in warmer, wetter conditions, Zhou et al. (2022) and Ishizaki et al. (2023) suggest that while increased tillering resulting from reduced *TB1* function does not boost yields in optimal conditions, it can enhance yield under water- or nutrient-limited stress. These studies are consistent with the hypothesis that lines with increased tillering plasticity can perform well in lower resource environments.

Potential Below Ground Effects

While increases in productive tiller number are observed in environments in which *tb-B1*-W341* single mutant lines yielded significantly higher than wildtype lines, these increases do not account for the overall magnitude of increase in grain yield. Lacking explanatory differences in above ground phenotype point towards differences in below ground phenotype such as increased root branching. Little to no decreases in grain size between genotypes despite other differences indicate that these mutant *TB1* genotypes may absorb more water and nutrients during grain fill. These observations contribute to the hypothesis that decreased *TB1* function increases root branching and biomass, similar to the conclusions of maize experiments in Gaudin et al. (2014). Increased root biomass can allow crops to access more water and belowground nutrients, increasing grain yields (Williams et al., 2022). Modern maize genotypes are associated with a decrease in root branching and root biomass compared to ancestral genotypes (Schmidt et al., 2016). Since maize yield is primarily limited by sunlight, this decrease in roots is not detrimental. However when wheat is grown under water limited conditions, deeper more highly branched rooting systems are associated with increases in grain yield (El Hassouni et al., 2018; Wasson et al., 2012; Zhu et al., 2018). Overall, if *TB1* affects roots similarly in wheat and maize,

increased rooting resulting from decreased *TB1* expression in wheat could be contributing to increase grain yield in environments with erratic growing season rainfall. More studies are needed examining the root morphology of different *TB1* genotypes in wheat.

Conclusion

Decreasing *TB1* function in durum wheat by introducing a single knockout allele *tb-B1*-W341* increases yields in some environments while maintaining similar yields to wildtype in other environments. In this study, reduced *TB1* function showed the greatest benefit in environments experiencing some drought stress, but also with some intermittent rainfall or supplemental irrigation. Because of their increased tillering plasticity, these lines can most effectively take advantage of mid-season irrigation or rainfall relative to wildtype, increasing productivity based on available moisture. If conditions are right, introduction of *tb-B1*-W341* can increase grain yield by up to 20 % without decreasing protein content. Further research is needed across more growing conditions, in other backgrounds, and on the root morphology of these genotypes to determine exactly how and when this increased tillering plasticity can improve grain yield in durum wheat.

References

Agriculture and Agri-Food Canada (AAFC) (2024). *Canada: Outlook for Principal Field Crops*. A. a. A.-F. Canada. https://agriculture.canada.ca/en/sector/crops/reports-statistics/canada-outlook-principal-field-crops-2024-12-19

Aguilar-Martinez, J. A., Poza-Carrion, C., & Cubas, P. (2007). Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. *Plant Cell*, *19*(2), 458-472. https://doi.org/10.1105/tpc.106.048934

Alqudah, A. M., Koppolu, R., Wolde, G. M., Graner, A., & Schnurbusch, T. (2016). The Genetic Architecture of Barley Plant Stature. *Frontiers in Genetics*, *7*. https://doi.org/10.3389/fgene.2016.00117

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

Bazargani, M. M., Sarhadi, E., Bushehri, A.-A. S., Matros, A., Mock, H.-P., Naghavi, M.-R.,…Moradi, F. (2011). A proteomics view on the role of drought-induced senescence and oxidative stress defense in enhanced stem reserves remobilization in wheat. *Journal of proteomics*, *74*(10), 1959-1973. https://doi.org/10.1016/j.jprot.2011.05.015

Borrill, P., Ramirez-Gonzalez, R., & Uauy, C. (2016). expVIP: a Customizable RNA-seq Data Analysis and Visualization Platform *Plant Physiology*, *170*(4), 2172-2186. https://doi.org/10.1104/pp.15.01667

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

Clements, R., Cross, R., & Sanders, P. (1974). Effect of sowing rate on the growth and yield of standard and semidwarf wheat cultivars. *New Zealand journal of experimental agriculture*, *2*(2), 139-144. https://doi.org/10.1080/03015521.1974.10425750

Cook, J., Acharya, R., Martin, J., Blake, N., Khan, I., Heo, H. Y.,…Sherman, J. (2021). Genetic analysis of stay-green, yield, and agronomic traits in spring wheat. *Crop Science*, *61*(1), 383-395. https://doi.org/10.1002/csc2.20302

Dixon, L. E., Greenwood, J. R., Bencivenga, S., Zhang, P., Cockram, J., Mellers, G., . . . Boden, S. A. (2018). TEOSINTE BRANCHED1 Regulates Inflorescence Architecture and Development in Bread Wheat (Triticum aestivum). *Plant Cell*, *30*(3), 563-581. https://doi.org/10.1105/tpc.17.00961

Dixon, L. E., Pasquariello, M., & Boden, S. A. (2020). TEOSINTE BRANCHED1 regulates height and stem internode length in bread wheat. *Journal of Experimental Botany*, *71*(16), 4742-4750. https://doi.org/10.1093/jxb/eraa252

Doebley, J., Stec, A., & Gustus, C. (1995). Teosinte Branched1 and the Origin of Maize - Evidence for Epistasis and the Evolution of Dominance. *Genetics*, *141*(1), 333-346. https://doi.org/10.1093/genetics/141.1.333

Doebley, J., Stec, A., & Hubbard, L. (1997). The evolution of apical dominance in maize. *Nature*, *386*(6624), 485-488. https://doi.org/10.1038/386485a0

Donald, C. (1981). 14 COMPETITIVE PLANTS, COMMUNAL PLANTS, AND YIELD IN WHEAT CROPS. *Wheat science-today and tomorrow*, 223.

Donald, C. t. (1968). The breeding of crop ideotypes. *Euphytica*, *17*, 385-403. https://doi.org/10.1007/BF00056241

Dong, B., Zheng, X., Liu, H., Able, J. A., Yang, H., Zhao, H.,…Liu, M. (2017). Effects of drought stress on pollen sterility, grain yield, abscisic acid and protective enzymes in two winter wheat cultivars. *Frontiers in Plant Science*, *8*, 1008. https://doi.org/10.3389/fpls.2017.01008

Duggan, B., Richards, R., Van Herwaarden, A., & Fettell, N. (2005). Agronomic evaluation of a tiller inhibition gene (tin) in wheat. I. Effect on yield, yield components, and grain protein. *Australian Journal of Agricultural Research*, *56*(2), 169-178.

El Baidouri, M., Murat, F., Veyssiere, M., Molinier, M., Flores, R., Burlot, L.,…Salse, J. (2017). Reconciling the evolutionary origin of bread wheat (Triticum aestivum). *New Phytologist*, *213*(3), 1477-1486. https://doi.org/10.1111/nph.14113

El Hassouni, K., Alahmad, S., Belkadi, B., Filali-Maltouf, A., Hickey, L. T., & Bassi, F. M. (2018). Root System Architecture and Its Association with Yield under Different Water Regimes in Durum Wheat. *Crop Science*, *58*(6), 2331-2346. https://doi.org/10.2135/cropsci2018.01.0076

Gaudin, A. C. M., McClymont, S. A., Soliman, S. S. M., & Raizada, M. N. (2014). The effect of altered dosage of a mutant allele of Teosinte branched 1 (tb1-ref) on the root system of modern maize. *Bmc Genetics*, *15*. https://doi.org/10.1186/1471-2156-15-23

Gonzalez-Grandio, E., Pajoro, A., Franco-Zorrilla, J. M., Tarancon, C., Immink, R. G. H., & Cubas, P. (2017). Abscisic acid signaling is controlled by a BRANCHED1/HD-ZIP I cascade in Arabidopsis axillary buds. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(2), E245-E254. https://doi.org/10.1073/pnas.1613199114

Hogg, A. C., Hale, C. O., Tillett, B. J., Huang, L., Carr, P. M., Eberly, J.,…Lamb, P. (2025). Registration of 'MT Blackbeard'and 'MT Raska'durum wheat. *Journal of Plant Registrations*, *19*(1), e20425. https://doi.org/10.1002/plr2.20425

Ishag, H., & Taha, M. (1974). Production and survival of tillers of wheat and their contribution to yield. *The Journal of Agricultural Science*, *83*(1), 117-124. https://doi.org/10.1017/S0021859600047079

Jafari, F., Wang, B., Wang, H., & Zou, J. (2024). Breeding maize of ideal plant architecture for high-density planting tolerance through modulating shade avoidance response and beyond. *Journal of Integrative Plant Biology*, *66*(5), 849-864. https://doi.org/10.1111/jipb.13603

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

Jones, B. H., Blake, N. K., Heo, H. Y., Martin, J. M., Torrion, J. A., & Talbert, L. E. (2021). Allelic response of yield component traits to resource availability in spring wheat.

Theoretical and Applied Genetics, *134*(2), 603-620. https://doi.org/10.1007/s00122-020-03717-7

Krasileva, K. V., Vasquez-Gross, H. A., Howell, T., Bailey, P., Paraiso, F., Clissold, L.,…Dubcovsky, J. (2017). Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(6), E913-E921. https://doi.org/10.1073/pnas.1619268114

Kukal, M. S., & Irmak, S. (2018). US agro-climate in 20th century: Growing degree days, first and last frost, growing season length, and impacts on crop yields. *Scientific reports*, *8*(1), 6977. https://doi.org/10.1038/s41598-018-25212-2

Lanning, S., Martin, J., Stougaard, R., Guillen-Portal, F., Blake, N., Sherman, J.,…Carlson, G. (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

Lewis, J. M., Mackintosh, C. A., Shin, S., Gilding, E., Kravchenko, S., Baldridge, G., . . . Muehlbauer, G. J. (2008). Overexpression of the maize Teosinte Branched1 gene in wheat suppresses tiller development. *Plant Cell Reports*, *27*(7), 1217-1225. https://doi.org/10.1007/s00299-008-0543-8

Liu, Y., Zhang, P., Li, M., Chang, L., Cheng, H., Chai, S., & Yang, D. (2020). Dynamic responses of accumulation and remobilization of water soluble carbohydrates in wheat stem to drought stress. *Plant Physiology and Biochemistry*, *155*, 262-270. https://doi.org/10.1016/j.plaphy.2020.07.024

Mock, J., & Pearce, R. (1975). An ideotype of maize. *Euphytica*, *24*(3), 613-623. https://doi.org/10.1007/BF00132898

Mueller, B., Hauser, M., Iles, C., Rimi, R. H., Zwiers, F. W., & Wan, H. (2015). Lengthening of the growing season in wheat and maize producing regions. *Weather and Climate Extremes*, *9*, 47-56. https://doi.org/10.1016/j.wace.2015.04.001

Naruoka, Y., Talbert, L. E., Lanning, S. P., Blake, N. K., Martin, J. M., & Sherman, J. D. (2011). Identification of quantitative trait loci for productive tiller number and its relationship to agronomic traits in spring wheat. *Theoretical and Applied Genetics*, *123*(6), 1043-1053. https://doi.org/10.1007/s00122-011-1646-0

Nasseer, A., Martin, J., Heo, H., Blake, N., Sherman, J., Pumphrey, M.,…Talbert, L. (2016). Impact of a quantitative trait locus for tiller number on plasticity of agronomic traits in spring wheat. *Crop Science*, *56*(2), 595-602. https://doi.org/10.2135/cropsci2015.05.0325

Peterson, J. (December 23, 2024). WMR-World Durum 2024. North Dakota Wheat Commission. https://www.ndwheat.com/post/wmr-world-durum-2024

Ramsay, L., Comadran, J., Druka, A., Marshall, D. F., Thomas, W. T. B., Macaulay, M., . . . Waugh, R. (2011). INTERMEDIUM-C, a modifier of lateral spikelet fertility in barley, is

an ortholog of the maize domestication gene TEOSINTE BRANCHED 1. *Nature Genetics*, *43*(2), 169-U125. https://doi.org/10.1038/ng.745

Richards, R. (1988). A tiller inhibitor gene in wheat and its effect on plant growth. *Australian Journal of Agricultural Research*, *39*(5), 749-757. https://doi.org/10.1071/AR9880749

Schmidt, J. E., Bowles, T. M., & Gaudin, A. C. M. (2016). Using Ancient Traits to Convert Soil Health into Crop Yield: Impact of Selection on Maize Root and Rhizosphere Function. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00373

Sedgley, R. (1991). An appraisal of the Donald ideotype after 21 years. *Field Crops Research*, *26*(2), 93-112. https://doi.org/10.1016/0378-4290(91)90031-P

Semenov, M. A., & Stratonovitch, P. (2013). Designing high-yielding wheat ideotypes for a changing climate. *Food and Energy Security*, *2*(3), 185-196. https://doi.org/10.1002/fes3.34

Senapati, N., Stratonovitch, P., Paul, M. J., & Semenov, M. A. (2019). Drought tolerance during reproductive development is important for increasing wheat yield potential under climate change in Europe. *Journal of experimental botany*, *70*(9), 2549-2560. https://doi.org/10.1093/jxb/ery226

Shang, Y., Yuan, L., Di, Z. C., Jia, Y., Zhang, Z. L., Li, S. J., . . . Li, C. D. (2020). A CYC/TB1-type TCP transcription factor controls spikelet meristem identity in barley. *Journal of Experimental Botany*, *71*(22), 7118-7131. https://doi.org/10.1093/jxb/eraa416

Sigalas, P. P., Bennett, T., Buchner, P., Thomas, S. G., Jamois, F., Arkoun, M.,…Hawkesford, M. J. (2024). At the crossroads: strigolactones mediate changes in cytokinin synthesis and signalling in response to nitrogen limitation. *The Plant Journal*, *120*(1), 139-158. https://doi.org/10.1111/tpj.16976

Studer, A., Zhao, Q., Ross-Ibarra, J., & Doebley, J. (2011). Identification of a functional transposon insertion in the maize domestication gene tb1. *Nature Genetics*, *43*(11), 1160-U1164. https://doi.org/10.1038/ng.942

Takeda, T., Suwa, Y., Suzuki, M., Kitano, H., Ueguchi-Tanaka, M., Ashikari, M., . . . Ueguchi, C. (2003). The OsTB1 gene negatively regulates lateral branching in rice. *Plant Journal*, *33*(3), 513-520. https://doi.org/10.1046/j.1365-313X.2003.01648.x

Thomashow, M. F. (1998). Role of cold-responsive genes in plant freezing tolerance. *Plant physiology*, *118*(1), 1-8. https://doi.org/10.1104/pp.118.1.1

Tokatlidis, I. (2014). Addressing the yield by density interaction is a prerequisite to bridge the yield gap of rain-fed wheat. *Annals of Applied Biology*, *165*(1), 27-42. https://doi.org/10.1111/aab.12121

U.S. Department of Agriculture (USDA). (2024). Small grains 2024 summary. U.S. Department of Agriculture, Economics, Statistics, and Marketing Information System. https://usda.library.cornell.edu/concern/publications/5t34sj573

Volkman, M. M., Martin, J. M., Hogg, A. C., Wright, L., Hale, C., Carr, P. M., & Giroux, M. J. (2022). Durum wheat Teosinte Branched1 null mutations increase tillering. *Crop Science*, *62*(4), 1522-1530. https://doi.org/10.1002/csc2.20775

Wang, H. W., Seiler, C., Sreenivasulu, N., Wiren, V., & Kuhlmann, M. (2022). INTERMEDIUM-C mediates the shade-induced bud growth arrest in barley. *Journal of Experimental Botany*, *73*(7), 1963-1977. https://doi.org/10.1093/jxb/erab542

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S. C., Prasad, S. V. S., Rebetzke, G. J., . . . Watt, M. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany*, *63*(9), 3485-3498. https://doi.org/10.1093/jxb/ers111

Whan, B., Delane, R., & Gilmour, R. (1988). The potential of reduced tillering wheats in dry environments. Proceedings of the seventh international wheat genetics symposium, held at Cambridge, UK, 13-19 July 1988.

Whipple, C. J., Kebrom, T. H., Weber, A. L., Yang, F., Hall, D., Meeley, R., Schmidt, R., Doebley, J., Brutnell, T.P., and Jackson, D. P. (2011). grassy tillers1 promotes apical dominance in maize and responds to shade signals in the grasses. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(33), E506-E512. https://doi.org/10.1073/pnas.1102819108

Williams, J. L., Sherman, J. D., Lamb, P., Cook, J., Lachowiec, J. A., & Bourgault, M. (2022). Relationships between roots, the stay-green phenotype, and agronomic performance in barley and wheat grown in semi-arid conditions. *The Plant Phenome Journal*, *5*(1), e220050. https://doi.org/10.1002/ppj2.20050

Xu, D. A., Bian, Y. J., Luo, X. M., Jia, C. F., Hao, Q. L., Tian, X. L.,…Cao, S. H. (2023). Dissecting pleiotropic functions of the wheat Green Revolution gene Rht1 in plant morphogenesis and yield formation. *Development*, *150*(20). https://doi.org/10.1242/dev.201601

Yunusa, I., & Sedgley, R. (1992). Reduced Tillering Spring Wheats for Heavy Textured Soils in a Semi-arid Mediterranean Environment. *Journal of Agronomy and Crop Science*, *168*(3), 159-168. https://doi.org/10.1111/j.1439-037X.1992.tb00994.x

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed research*, *14*(6), 415-421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zhu, Y. H., Ren, L. L., Horton, R., Lu, H. S., Wang, Z. L., & Yuan, F. (2018). Estimating the Contribution of Groundwater to the Root Zone of Winter Wheat Using Root Density

Distribution Functions. *Vadose Zone Journal*, *17*(1). https://doi.org/10.2136/vzj2017.04.0075

Zwirek, M., Waugh, R., & McKim, S. M. (2019). Interaction between row-type genes in barley controls meristem determinacy and reveals novel routes to improved grain. *New Phytol*, *221*(4), 1950-1965. https://doi.org/10.1111/nph.15548

CHAPTER SIX

INCREASING GRAIN YIELD AND INDUCING MULTI-ROW SPIKELET PHENOTYPE IN SPRING WHEAT THROUGH MUTATIONS IN *TEOSINTE BRANCHED-1*

Abstract

In hexaploid wheat (*Triticum aestivum* L.), reduced gene expression or protein function of *Teosinte Branched-1 (TB1)* increases tiller number while overexpression induces a supernumerary paired spikelet phenotype. This study examines spring wheat lines containing natural and artificially induced mutant alleles of *TB1*, with the hypothesis that increasing productive tiller number by decreasing *TB1* function will increase grain yield. Results demonstrate that the natural mutant allele *TB-D1b* can increase grain yields in dryland environments, although no significant differences in yield component traits were detected. More notedly, introgression of ethyl methanesulfonate (EMS) derived mutant alleles *tb-A1*-R256* and *tb-D1*-Q49* into the Montana adapted variety 'Duclair' markedly increased tiller number without decreasing spike size or single grain weight. This lead to significantly increased grain yield in field conditions without reducing grain protein content. Similar effects were seen in a 'Vida' background containing *tb-A1*-R256* alone, likely because Vida produces more tillers than Duclair to begin with. Additionally, a novel spike type displaying supernumerary 'multi-row spikelets' was observed in Vida and Duclair background lines containing mutant alleles of all three *TB1* homoeologs. This trait contrasts the known 'paired spikelet' phenotype resulting from

TB1 over-expression. This study supports introgression of natural and artificial mutant alleles of *TB1* into spring wheat breeding programs to increase productive tiller number and grain yield.

Introduction

Spring wheat (*Triticum aestivum* L.) production is a central component of cropping systems in the Northern Great Plains region of the United States, with over 82 % of national spring wheat production occurring in North Dakota, Minnesota, and Montana (USDA, 2024). These regions experience variable and often harsh environmental conditions ,presenting challenges for crop breeders. In this region it is important to develop varieties that are heat and drought tolerant to help farmers produce a harvestable crop during poor years. Additionally, it is equally important for breeders to develop varieties with high yield potential enabling high grain yields if conditions are favorable. Breeders can achieve this by selecting for higher tillering potential: the ability to produce more tillers depending on experienced environmental conditions. In Montana, lines with higher tillering potential outperform sister lines in relatively higher resource conditions (Jones et al., 2021; Naruoka et al., 2011; Nasseer et al., 2016).

The gene *Teosinte Branched-1 (TB1)* directly affects tillering in wheat and is an ideal candidate for modification to improve tillering potential. This gene was first described by Doebley et al. (1995) as a master regulator of tillering in maize *(Zea mays* L. subsp. *mays)*, with upregulation of *TB1* being a key contributor to the single stalk phenotype of modern maize relative to the bushy phenotype of its wild ancestor teosinte *(Zea mays* L. subsp. *parviglumis)*. Orthologs have been identified in many other plant species, referred to as *Teosinte Branched-1 (TB1)* in monocots but as *Branched-1 (BRC1)* in dicots (Wang et al., 2019). Overexpression of *TB1* in wheat decreases tiller number and increases instances of a paired spikelet phenotype

(Dixon et al., 2018; Dixon et al., 2020; Lewis et al., 2008). Previous studies in tetraploid durum wheat *(Triticum turgidum* L. subsp. *durum)* have shown increases in tiller number in lines containing one or two nonsense mutation alleles (Volkman et al., 2022, Chapter 5). However, no studies have directly examined reduced function *TB1* genotypes in hexaploid wheat. Dixon et al. (2018) primarily focused on lines with *TB1* overexpression and demonstrated that overexpression led to decreased tillering and increased instances of a paired spikelet phenotype. This study also describes two naturally occurring mutant alleles of *TB1* in wheat, one in the B genome referred to as *TB-B1b*, and one in the D genome referred to as *TB-D1b*. The *TB-B1b* mutation is characterized by two silent mutations and three missense mutations resulting in three amino acid changes, each change with an individual PROVEAN score of between zero and -1.1 (not predicted to be deleterious individually) (Choi & Chan, 2015). The *TB-D1b* mutation is characterized by only one amino acid change, however this mutation occurs within the conserved TCP region and has a PROVEAN score of -7.9 (predicted to be deleterious). Data from Dixon et al. (2018) suggests that natural allelic variation of *TB1* may affect tillering and inflorescence architecture (specifically decreased instances of paired spikelets). However, differences in tillering and paired spikelet occurrences were only shown relative to *TB1* overexpression lines. It was further hypothesized in Dixon et al. (2020) that variant alleles that alter *TB1* expression or function could be used in breeding programs to optimize wheat plant height and tillering. To test this, Volkman et al. (2022) screened a set of 40 Montana adapted wheat varieties for *TB1* allelic variation. The screen included 15 spring wheat varieties, 15 winter wheat varieties, and 10 durum varieties. This screen showed no variation in durum or winter wheat, but that spring wheat lines ‘McNeal’ (PI 574642) and ‘Egan’ (PI 671855) contained the *TB-B1b* mutant allele, while

‘Choteau’ (PI 633974), ‘Duclair’ (PI 660981), ‘NS Presser’ (PI 679964), and ‘Vida’ (PI 642366) contained the *TB-D1b* mutant allele (Blake et al., 2014; Heo et al., 2018; Lanning et al., 2011; Lanning et al., 2004; Lanning et al., 1994; Lanning et al., 2006).

This study first makes comparisons between the natural mutant *TB-D1b* and wildtype *TB-D1a* alleles in a recombinant inbred line (RIL) population over two seasons to test for the effects of this natural mutant allele in Montana. Due to logistical constraints involving linked *Reduced Height-1 (Rht-1)* semi-dwarfing alleles, comparisons were not made between *TB-B1b* and *TB-B1a* alleles. Positive results from the RIL study encouraged further investigation into artificially induced *TB1* mutant alleles to further enhance tillering and grain yield. Linkages between *Rht-1* and *TB1* were closely examined, as this linkage affects the feasibility of creating experimental lines to test natural *TB1* mutant alleles in non-confounding comparisons. Considering *TB1* and *Rht-1* linkage constraints, a scheme was designed to create uniformly semidwarf near isogenic lines (NIL) in two different Montana adapted backgrounds to test combinations of mutant alleles of all three hexaploid wheat *TB1* homoeologs. NILs were grown in full density yield trials in summer 2024 to assess tiller number, unique spike morphology traits, and overall agronomic performance.

Materials and Methods

TB1 and *Rht-1* Linkage Analysis

The three hexaploid wheat *TB1* and *Rht-1* homoeologs were located in the IWGSC RefSeq v1.0 genome using the Plant Ensembl browser and gene identification numbers were cross validated (Bolser et al., 2017). Physical locations of these genes in relation to each other were noted, as well as their relative location on each of the group 4 chromosomes.

Recombination frequencies between each pair of homoeologs were calculated using genetic distance estimations from Gutierrez-Gonzalez et al. (2019) which used dense genotyping by sequencing markers to create maps estimating recombination frequencies across the wheat genome. In this way, estimated genetic distances between the three respective *TB1* and *Rht-1* homoeologs were calculated to determine the likelihood of these homoeologs ever breaking linkage. These estimations also informed the practicality of using linked markers for genotyping.

RIL Experiment Investigating Natural Alleles

An RIL population that varied for wild type (*TB-D1a*) and mutant (*TB-D1b*) D genome alleles was analyzed over two years. This population was developed from a cross between a spring habit version of the Montana hard red winter wheat variety 'Yellowstone' (PI 643428) called 'Spring Yellowstone' and the hard red spring wheat variety Vida (Bruckner et al., 2007; Cook et al., 2018; Lanning et al., 2006). Spring Yellowstone contains the wildtype *TB-D1a* allele and Vida contains the natural mutant *TB-D1b* allele. Both lines are semidwarf and contain the *Rht-B1b* allele on the B genome. Seed from the F_1 cross between these lines was advanced to the F_5 generation by single seed decent and planted in individual head rows. This population has been used to study other segregating natural alleles as well. Additional details on this population and field study can be found in Hale et al. (2024) (Chapter 2).

One hundred forty-six $F_{5:6}$ lines were planted in two randomized complete block design experiments, each with two replications. One experiment was planted in an irrigated environment and one experiment in a separate but adjacent rainfed environment. Seventy-three lines contained the mutant allele *TB-D1b*, 64 contained the wildtype allele *TB-D1a*, and four were heterozygous. This experiment was planted in 2021 and 2022 at the Post Agronomy Farm

near Bozeman, MT. Seeding rate for this experiment was approximately 297 seeds per m^2. In 2021, experiments were planted on 21 April. , This location received 20.0 cm of precipitation between 1 April and 31 August. The 2021 irrigated experiment was watered on 22 and 28 June and 5 July with approximately 5 cm of irrigation water (for a total of 15 cm) using hand line sprinklers. In 2022, experiments were planted on 6 May and received 24.0 cm of precipitation between 1 April and 31 August. The 2022 irrigated experiment again received a total of 15 cm of irrigation water applied in 5 cm increments on 21 and 26 June, and 2 July. The 2021 field season was hotter than average , with average monthly temperatures in April, May, June, July, and August of 41.5, 48.6, 64.9, 73.7, and 66.1 °C respectively, while average monthly growing season temperatures in 2022 were 37.2, 48.4, 59.7, 69.0, and 71.0 °C respectively (NCEI, 2025).

EMS induced *TB1* Mutant Allele NIL Development

To create spring wheat lines with varied levels of decreased *TB1* function, four different deleterious mutant alleles were selected for combination with a near isogenic line (NIL) population. *TB1* mutations from mutagenized populations of ‘Cadenza’ and ‘Alpowa’ (PI 566595) were used to create backcrossed NILs in Vida and Duclair backgrounds. Both Vida and Duclair contain the natural *TB-D1b* mutant allele, so any increase in tillering and grain yield relative to the wildtype would be on top of any advantages realized from *TB-D1b*. Both varieties are modern, high yielding, high quality hard red spring wheat varieties. They both contain the semidwarfing allele *Rht-B1b*. Vida is a hollow stemmed line known to produce a relatively high number of tillers. Duclair is a solid stemmed sawfly resistant variety generally producing fewer tillers than Vida (Lanning et al., 2011; Lanning et al., 2006).

Alleles *tb-A1*-R256* (CGA to TGA) in the line ‘Cadenza667’, and *tb-D1*-Q49* (CAA to TAA) in the line ‘Cadenza1721’ were obtained from the Cadenza TILLING population (Krasileva et al., 2017). Cadenza is a European hard red wheat with a facultative winter growth habit and a tall phenotype, containing both *Rht-B1a* and *Rht-D1a* wildtype alleles. Alleles *tb-B1*-R127W (CGG to TGG) and *tb-D1*-R125W (CGG to TGG) were obtained from an ethyl methanesulfonate (EMS) mutagenized population of Alpowa developed at Montana State University (Feiz et al., 2009). Alpowa is a soft white spring wheat with a semidwarf phenotype conferred by the *Rht-D1b* semidwarfing allele on the D genome. Missense mutation alleles *tb-B1*-R127W and *tb-D1*-R125W have a PROVEAN score of -7.8 and -7.9 respectively, with a high chance of being deleterious (Choi & Chan, 2015). Because of their low PROVEAN scores, these two missense mutant alleles were presumed to confer similar knockout of function effects as nonsense mutant alleles *tb-A1*-R256* and *tb-D1*-Q49*. Derived from Alpowa, the *tb-B1*-R127W mutant allele is linked to an *Rht-B1a* wild type allele. In fact, all known *TB-B1* mutant alleles (including the natural mutant allele *TB-B1b*) are linked to the wildtype *Rht-B1a* allele. Similarly, the natural mutant allele *TB-D1b* is linked to *Rht-D1a* and has only been found to exist either in tall lines or in semidwarf lines that contain the unlinked *Rht-B1b* allele (Dixon et al., 2020; Volkman et al., 2022). The *tb-d1*-Q49* mutant allele is linked to an *Rht-D1a* wild type allele, and the *tb-D1*-R125W mutant allele is linked to the *Rht-D1b* semidwarfing allele. The *tb-D1*-R125W mutant allele is the only known *TB1* mutant allele linked to a semidwarfing *Rht-1* allele. Utilizing these two nonsense mutant *TB-D1* alleles linked to different *Rht-D1* alleles, NIL that vary for *TB1* mutations but that have a semidwarf phenotype were created in the backgrounds Vida and Duclair (Table 6.1). Past studies on *Rht-D1b* and *Rht-B1b* alleles have determined their

effects to be equivalent, so lines with either allele are considered to have an equivalent semidwarf phenotype (Jobson et al., 2018; Lanning et al., 2012; Li et al., 2006).

Table 6.1. Genotypes of selected semidwarf Near Isogenic Lines (NIL) varying for *TB1* missense alleles. Bolding indicates loci that confer a semidwarf phenotype. Exactly one *Rht-1* semidwarfing allele (*Rht-B1b* or *Rht-D1b*) must be present to confer a semidwarf phenotype.

Overall *TB1* Genotype	**Gene**	**A Genome Alleles**	**B Genome Alleles**	**D Genome Alleles**
Wildtype	*TB1* *Rht-1*	*TB-A1a* *Rht-A1a*	***TB-B1a*** ***Rht-B1b***	*TB-D1b* *Rht-D1a*
Single *(TB-A1)* mutant	*TB1* *Rht-1*	*tb-A1*-R256* *Rht-A1a*	***TB-B1a*** ***Rht-B1b***	*TB-D1b* *Rht-D1a*
Single *(TB-D1)* mutant	*TB1* *Rht-1*	*TB-A1a* *Rht-A1a*	***TB-B1a*** ***Rht-B1b***	*tb-D1*-Q17* *Rht-D1a*
Double (*TB-A1* and *TB-D1*) mutant	*TB1* *Rht-1*	*tb-A1*-R256* *Rht-A1a*	***TB-B1a*** ***Rht-B1b***	*tb-D1*-Q17* *Rht-D1a*
Double (*TB-B1* and *TB-D1*) mutant	*TB1* *Rht-1*	*TB-A1a* *Rht-A1a*	*tb-B1*-R127W *Rht-B1a*	***tb-D1*-R125W** ***Rht-D1b***
Triple (*TB-A1, TB-B1* and *TB-D1*) mutant	*TB1* *Rht-1*	*tb-A1*-R256* *Rht-A1a*	*tb-B1*-R127W *Rht-B1a*	***tb-D1*-R125W** ***Rht-D1b***

To create lines containing the combinations of alleles shown in Table 6.1, source lines containing single mutant alleles were crossed together, and then backcrossed into Vida and Duclair. Two different crossing schemes in each background were created, one using *tb-D1*-Q49*,* the other using *tb-D1*-R125W. First, Cadenza667 with the *tb-A1*-R256* allele was crossed to Cadenza1721 containing the *tb-D1*-Q49* allele and to Alpowa background plants containing the *tb-D1*-R125W allele. F_1 plants from these two different crosses were then crossed to Alpowa background plants containing the *tb-B1*-R127W allele. Progeny from these two different three-way crosses were then crossed to both Vida and Duclair. Lines were backcrossed respectively to Vida or Duclair either four or five times. At each generation, progeny were genotyped to ensure

retention of each *TB1* mutant allele. Lines containing *tb-A1*-R256*, tb-B1*-R127W, and *tb-D1*-R125W mutant alleles were backcrossed until they reached the BC_4F_1 generation. Lines containing the *tb-A1*-R256*, tb-B1*-R127W, and *tb-D1*-Q49* mutant alleles were backcrossed to the BC_5F_1 generation. Single plants heterozygous for each of their respective mutant alleles were selected to generate NIL populations. Six hundred F_2 seeds were planted from each of the four populations, and lines were advanced by single seed decent to the F_5 generation. In summer 2023, seeds were planted in the field in single spaced plant rows with 15 cm between each plant. Two hundred fifty-six F_5 plants were grown from the Duclair background population segregating for the *tb-A1*-R256*, tb-B1*-R127W, and *tb-D1*-Q49* alleles; 348 F_5 plants were grown from the Vida background population segregating for the *tb-A1*-R256*, tb-B1*-R127W, and *tb-D1*-Q49* alleles; 186 F_5 plants were grown from the Duclair background population segregating for the *tb-A1*-R256*, tb-B1*-R127W, and *tb-D1*-R125W alleles; and 197 F_5 plants were grown from the Vida background population segregating for the *tb-A1*-R256*, tb-B1*-R127W, and *tb-D1*-R125W alleles. DNA samples were collected from each plant and genotyped for each allele. Plants that were homozygous at each *TB1* locus were selected as NIL. In this way, semidwarf NIL representing six of the eight possible *TB1* allele combinations were created in two different backgrounds (Table 6.1). Since there are no known *TB1* mutant alleles linked to the *Rht-B1b* semidwarfing allele, there were no single mutant *tb-B1* genotypes or double mutant *tb-A1*/*tb-B1* genotypes generated from this crossing scheme. Generating these missing genotypes would have been possible but would have required a third crossing population including a wildtype *TB-D1a* allele linked to a semidwarfing *Rht-D1b* allele. At least nine different lines were generated representing each generated allele combination group. If more than 10 lines were generated

within a group, 10 lines were selected randomly to advance. All selected lines were harvested, and $F_{5:6}$ seed was sent to Yuma, AZ for seed increase over the winter of 2023-2024.

NIL Field Experiment Investigating EMS Derived Alleles

All selected NIL were planted at the Post Agronomy Farm near Bozeman, MT in 2024 randomized complete block design experiments including two replications in an irrigated environment and two replications in a separate but adjacent non-irrigated (rainfed) environment. Populations representing the two backgrounds (Vida and Duclair) were planted in two separate randomized blocks within each replicate. All isolines representing the six different genotypes were completely randomized within each block. Each block included four parent variety checks. Rainfed plots were planted on 16 May and Irrigated plots were planted on 17 May. Irrigated plots were given 5 cm of supplemental water on 5 July using hand line sprinklers. Plots consisted of two 3.3 m long rows spaced 30 cm apart. Seeding rate for this experiment was approximately 224 seeds per m^2. Between 1 April and 31 August, this location received 20.3 cm of precipitation, and monthly average temperatures for April, May, June, July, and August were 45.4, 49.1, 61.2, 68.7, and 65.9 °C respectively (NCEI, 2025).

Genotyping Methods

Screening RIL for the *TB-D1b* allele in the Vida/Spring Yellowstone population was performed by sequencing as in Volkman et al. (2020). Backcrossing of the *tb-A1*-R256*, *tb-B1*-R127W, *tb-D1*-Q49*, and *tb-D1*-R125W alleles into Vida and Duclair was performed using allele specific qPCR PACE® genotyping markers (3CR Bioscience, UK). The *tb-B1*-R127W and *tb-D1*-R125W alleles are derived from Alpowa containing the semi dwarfing allele *Rht-D1b*. The *tb-B1*-R127W allele is linked to *Rht-B1a*, and the *tb-D1*-R125W allele is linked to *Rht-D1b* (see

Tables 6.1 and 6.3). Vida and Duclair both contain the semidwarfing allele *Rht-B1b*, and the wildtype *Rht-D1a* allele. Since *Rht-1* and *TB1* are closely linked, and because the recurrent parents contain different *Rht-1* alleles than the donor line, published allele specific qPCR assays developed for *Rht-1* alleles were used to select for linked *TB1* mutant alleles (Rasheed et al., 2016). In Vida and Duclair recurrent parent backgrounds, lines containing *Rht-D1b* were presumed to contain *tb-D1*-R125W, and lines containing *Rht-B1a* were presumed to contain *tb-B1*-R127W. The recombination frequency between the *Rht-1* marker alleles and the *TB1* alleles was predicted to be 1:400 or 1:600 for the B and D genomes respectively, so although highly unlikely, recombination was possible (Table 6.3). Therefore, genotyping by sequencing as in Volkman et al. (2022) was additionally applied to a subset of lines during the backcrossing process to ensure that no crossover events had occurred between these two loci.

Allele specific qPCR PACE® assays were developed to genotype for the two Cadenza TILLING population derived mutations, *tb-A1*-R256* and *tb-D1*-Q49*. Primers developed for these two assays are listed in Table 6.2. Each reaction contained 3.66 µl of ultrapure nuclease free water, 5 µl of PACE® master mix, 0.14 µl of pre mixed three primer stock, and 1.2 µl of genomic DNA at a concentration of about 2 µg per µl. One hundred microliter batches of pre mixed primer stock were prepared by mixing 12 µl of 100 mM HEX tagged primer, 12 µl of 100 mM FAM tagged primer, and 30 µl of 100 mM common primer in 46 µl of 10 mM Tris buffer with a pH of 8.3. PCR conditions involved a touchdown PCR protocol with an initial denaturation at 94 °C for 15 min followed by cycles of denaturation at 94 °C for 20 sec with a combined annealing and extension of 65 °C for 1 min that decreased by 0.8 °C each cycle for 10

cycles. This touchdown sequence was followed by 29 cycles of 94 °C denaturation for 20 sec, 57 °C of combined annealing and extension for 1 min, and a plate read step at 30 C for 30 sec.

Table 6.2. List of primers developed to perform allele specific qPCR PACE® assays to genotype for the Cadenza TILLING population derived alleles *tb-A1-R256** and *tb-D1-Q49**.

Allele	Primer Name	Primer Sequence
*tb-A1-R256**	Tb1A 667 Hex For (wt)	5' HEX-CGGGAAAAGAACCGGATGC 3'
	Tb1A 667 Fam For (mut)	5' FAM-CGGGAAAAGAACCGGATGT 3'
	Tb1A Rev 2	5' CGAATGCAGGGGAGGAATGA 3'
*tb-D1-Q49**	Tb1D 1721 Hex For (wt)	5' HEX-CATGGACTTGCCCCTTTACC 3'
	Tb1D 1721 Fam For (mut)	5' FAM-CATGGACTTGCCCCTTTACT 3'
	Tb1D Rev2	5' CGAATGCAGGGGAGGAATGA 3'

Phenotyping Methods

For all experiments, heading date was recorded for each plot in days from January 1 on which 50 % of the spikes in each plot had fully emerged from the sheath. Physiological maturity was recorded for each plot in days from January 1 on which 50 % of peduncles in the plot had turned brown. After physiological maturity, tillers and productive spikes were counted within a representative 30 cm section of row. Tillers and productive spikes were counted in all reps of all experiments, except for the second replication of the rainfed Vida/Spring Yellowstone RIL experiment in 2021, and the second replication of both rainfed and irrigated experiments in 2022. Height was measured in two representative locations in each plot as the length from the surface of the soil to the tip of a representative spike, not including awns. Spikelets per spike were counted on three representative primary spikes in each plot. Primary spikes were defined as those that were be = larger, ands more mature relative to other spikes in the plot. In the NIL experiments, presence or absence of a multi-row spikelet phenotype was recorded for each plot, and photographs were taken of representative spike samples in each variety background. All

plots were harvested with a small plot combine, and harvested grain was weighed to determine equivalent grain yield. After harvest, grain protein content was measured by near-infrared transmittance using a Foss Infratec 1241 Grain Analyzer (Foss North America, Silver Springs, MD) and seed size was measured by weighing a sample of 200 seeds from cleaned subsamples of each harvested plot.

Statistical Analysis

Mixed model analysis of variance using the lme4 package (Bates et al., 2015) in R (R Foundation for Statistical Computing. Version 4.0.5. Vienna, Austria) was used to analyze data from all experiments. For the Vida/Spring Yellowstone RIL population data, each year by experiment within year combination was treated as an individual environment. The model used included environment, *TB-D1* allele class, and interaction between *TB-D1* and the environment, which were considered fixed effects. Additionally, the model included lines within *TB-D1* allele class, and block within year which were considered as random effects. To analyze data from the 2024 spring wheat *TB1* NIL experiment, mean response variable values of each NIL genotype group within each replication were calculated, and these mean values were analyzed via mixed model analysis of variance. Data from each of the two backgrounds was analyzed separately. There were two different environments (rainfed and irrigated). *TB1* genotype, the environment, and their interaction were considered as fixed effects, while replicate within environment was considered as a random effect.

Results

TB1 and *Rht-1* Linkage Results

TB1 and *Rht-1* homoeologs on the three group four chromosomes were found to occur at similar physical distances from each other. However, the homoeologs on chromosome 4A were on the opposite arm relative to 4B and 4C chromosomes, and closer to the centromere resulting in closer genetic distance and tighter linkage (Table 6.3).

Table 6.3. Physical and genetic distances between *TB1* and *Rht-1* homoeologs on each of the group 4 chromosomes. Genetic location information retrieved from plant ensembl October 2024. [a]cM/Mb estimates based on data from Gutierrez-Gonzalez et al. (2019)

Gene	ID	Chromosome	Physical Location Start	Physical Location End	Distance apart	Relative position from centromere	Recom-bination Frequency Estimate [a]	Genetic Distance Estimate
			basepairs	basepairs	basepairs	%	cM/Mb	cM
TB-A1	TraesCS4A02G271300	4A	582,839,670	582,841,244	360,092	33.6	<0.1	<0.03
Rht-A1	TraesCS4A02G271000		582,477,351	582,479,578				
TB-B1	TraesCS4B02G042700	4B	30,362,277	30,363,341	497,927	90.4	0.5	0.25
Rht-B1	TraesCS4B02G043100		30,861,268	30,863,723				
TB-D1	TraesCS4D02G040100	4D	18,463,838	18,472,387	308,675	90.0	0.5	0.16
Rht-D1	TraesCS4D02G040400		18,781,062	18,782,933				

Natural Allele RIL Experiment Results

Out of all measured parameters in the Vida/Spring Yellowstone RIL population, the only significant mean differences between lines containing the *TB-D1a* wildtype allele and the *TB-D1b* natural mutant allele were in overall grain yield. Mean grain yield was significantly higher in the *TB-D1b* natural mutant allele lines across both years in the rainfed experiment, showing a 9.0 % increase ($p = 0.01$) in 2021, and a 3.6 % increase ($p = 0.03$) in 2022 (Supplemental Table 6.1). In irrigated environments, there were no significant differences in mean yield between the two allele classes. However, yields trended down for the *TB-D1b* natural mutant allele in 2021, but up in 2022 (Supplemental Table 6.1). This led to a significant genotype by environment interaction for yield, indicating that the natural mutant allele is more beneficial in rainfed environments than in irrigated environments. Seed size trended up and plant height trended down slightly for lines containing the *TB-D1b* natural mutant allele across all tested environments. No tiller count data from any of the replications showed trends in favor of either allele, which is why tiller counts were not conducted across all replicates each year. Additionally, no differences were seen between allele classes in mean heading date, maturity date, spikelets per spike, or grain protein content. See Table 6.4 for mean values of measured parameters across all environment/years and see supplementary Table D6.1 for mean values split up by environment and location.

Table 6.4. Vida by Spring Yellowstone RIL Population data averaged across 2021 and 2022 years, irrigated and rainfed environments. TB-D1b natural mutant group (76 RILs) compared with TB-D1a wildtype group (65 RILs).

***TB-D1* Allele**	**Heading Date**	**Maturity Date**	**Tillers**	**Prod Spikes**	**Leaf Length**	**Plant Height**
genotype	Julian	Julian	per ft	per ft	cm	cm
TB-D1b	186.7±0.2	222.4±0.21	779±24.2	684±29.6	17.5±0.35	88.0±0.65
TB-D1a	187±0.22	222.3±0.23	779±24.4	678±29.7	17.2±0.36	88.7±0.69
p from ANOVA						
TB-D1	0.41	0.82	0.93	0.71	0.38	0.44
Environment	< 0.0001	< 0.0001	0.004	0.11	< 0.0001	< 0.0001
GxE	0.78	0.24	0.71	0.74	0.53	0.77

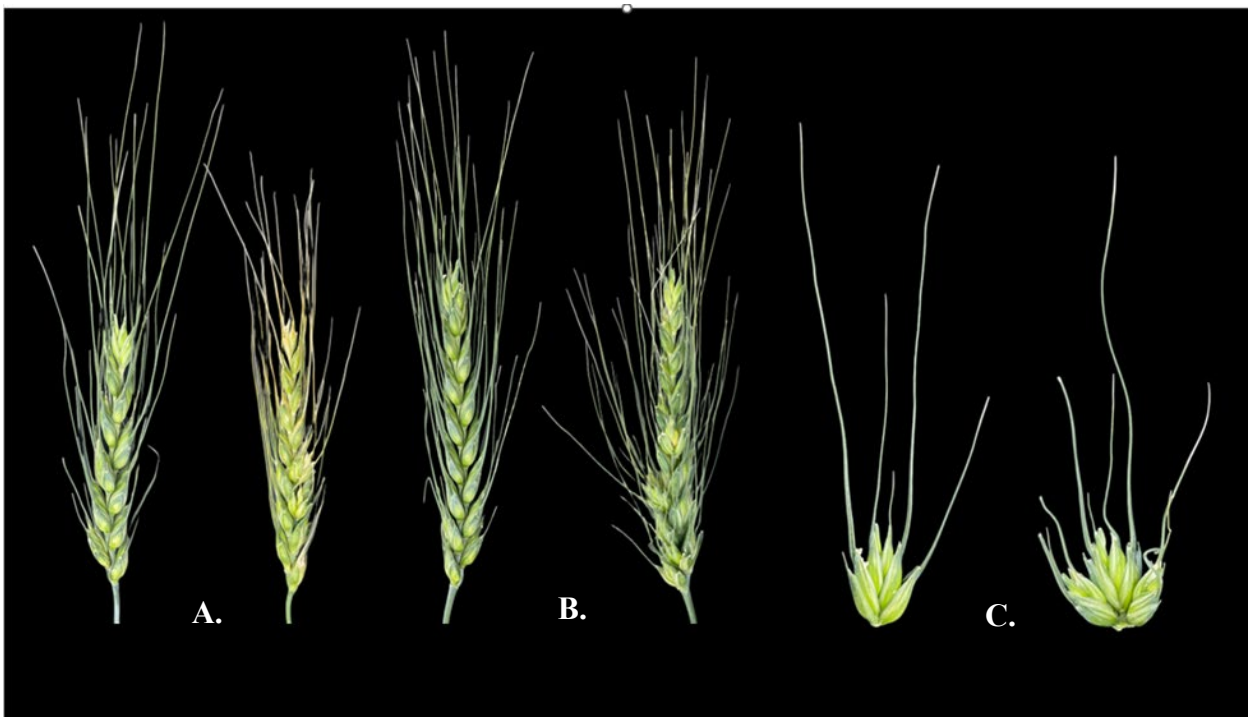
	Grain Yield	**Single Grain Wt**	**Grain Protein**	**Spikelets/ Spike**	**Yield/ Spike**	**Seeds/ Spikelet**
	kg ha^{-1}	mg	g kg^{-1}	no	g	no
TB-D1b	6263±83	30.3±0.30	139±0.65	17.5±0.15	1.57±0.02	2.80±0.03
TB-D1a	6144±86	29.8±0.32	139±0.68	17.5±0.16	1.55±0.02	2.81±0.03
p from ANOVA						
TB-D1	0.03	0.17	0.87	0.95	0.46	0.95
Environment	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0002
GxE	0.0001	0.19	0.018	0.23	0.0014	0.02

EMS induced Allele NIL Experiment Results

The triple *TB1* mutant genotype groups had many more tillers on average at maturity than the wildtype groups, with the Duclair background triple mutant group showing a 30.7 % increase in tillers (Table 6.5) and the Vida background triple mutant group showing a 22.5 % increase in tillers (Table 6.6). These increases in tiller number corresponded with reductions in single grain weight, which was the primary reason why the triple mutant lines did not yield more grain than the wildtype group (Figures 6.2-6.5). Triple mutant lines were shorter on average than wildtype lines, with Duclair background triple mutant lines showing a 4.3 cm height decrease on average (Table 6.5), and Vida background triple mutant lines showing a 5.6 cm height decrease on

average (Table 6.6). A novel supernumerary spikelet phenotype characterized as multi-row spikelets was observed in all lines with a triple *TB1* mutant genotype. This phenotype was characterized by three spikelets originating from the same rachis node on between two and five nodes in the middle to lower section of the rachis (Figure 6.1). This spike phenotype occurred at a frequency of about one spike per plant within each plot.

Figure 6.1. Multi-row spikelet phenotype observed in triple TB1 mutant allele NIL in both Vida and Duclair backgrounds. A. Spikes from Vida background NIL representing a fully wildtype genotype (left) and a triple mutant genotype (right). B. Spikes from Duclair background NIL representing a fully wildtype genotype (left) and a triple mutant genotype (right). C. Spikelets originating from a single rachis node, illustrating a standard spikelet (left) a multi-row spikelet phenotype with two supernumary spikelets forming laterally from a central spikelet (right).



Across genotype groups in the Duclair background NIL experiment, the *tb-A1*-R256* and *tb-D1*-Q49* double mutant allele combination yielded the highest, with a statistically significant average grain yield increase of 8.4 % compared to the wildtype group. This increase was the

result of a statistically significant increase in productive spikes of 16.2 % with no statistically significant reduction in either spikelets per spike or single grain weight (Table 6.5, Figure 6.2, and Figure 6.3). This genotype group showed an average height decrease of 1.6 cm when compared to the wildtype group, and did not show a statistically significant decrease in grain protein content. Across the *TB1* NIL genotype combinations in the Vida background, the *tb-A1*-R256* allele single mutant lines showed the highest average grain yields, producing 5.4 % more grain on average than the wildtype lines, although this specific difference was not statistically significant. This yield increase can be attributed to the statistically significant increase in productive spikes by 15.3 %, and only small reductions in spikelets per spike (2.3 %) and single grain weight (2.1 %) when compared to reductions in other genotype groups (Table 6.6, Figure 6.4 and Figure 6.5). The Vida background *tb-A1*-R256* allele single mutant lines did not show a significant difference in average grain protein content and were on average 2.3 cm shorter than the wildtype group. Across all measured traits in the NIL experiment, the Vida background NIL plant heights displayed the only significant genotype by environment interaction. Trends in the rank order of average plant height across genotype groups were similar across rainfed and irrigated environments, but differences were much more pronounced in the irrigated environment resulting in a significant interaction. Since rank order was similar, only mean values across both environments are reported (Table 6.6).

Table 6.5. Duclair background *TB1* induced mutant allele Near Isogenic Line (NIL) results. Lines containing *TB-A1*-R256*, *TB-B1a*, and *TB-D1*-Q17* alleles yielded significantly higher on average than wildtype lines and did not show a significant decrease in grain protein content. [a] Heading date was determined by the julian date on which >50% of spikes 100% out of boot. Highest values in each column are underlined, and the highest significant values are bolded according to calculated LSD values.

Duclair			**Grain Yield**	**Maturity Date[a]**	**Tillers at Maturity**	**Productive Spikes**	**Height**	**Spikelets/ Spike**	**Grain Protein Content**	**Single Grain Weight**
TB-A1	**TB-B1**	**TB-D1**	kg ha^{-1}	d	tillers m^{-2}	spikes m^{-2}	cm	no	g kg^{-1}	mg
wt	wt	wt (D1b)	4788	228.5	645	629	**77.5**	**14.2**	144	30.7
R256*	wt	wt (D1b)	4768	**229.6**	646	632	74.3	**14.5**	143	**31.3**
wt	wt	Q17*	4775	**229.2**	686	671	76.3	**14.4**	143	**30.8**
R256*	wt	Q17*	**5192**	**229.3**	**746**	**731**	75.9	**14.5**	143	30.2
wt	R127W	R125W	**4896**	229.0	**730**	**716**	73.8	13.6	**147**	30.5
R256*	R127W	R125W	4721	228.3	**833**	**793**	73.2	13.2	145	28.6
		LSD Value	324	0.57	66	60	1.11	0.39	1.2	0.60
		p from ANOVA								
		Genotype	0.013	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
		Environment	0.048	<0.001	<0.001	<0.001	0.347	0.771	0.206	0.040
		GxE	0.208	0.211	0.226	0.045	0.771	0.159	0.905	0.152

Table 6.6. Vida background *TB1* induced mutant allele Near Isogenic Line (NIL) results. Lines containing *TB-A1*-R256*, *TB-B1a*, and *TB-D1a* alleles yielded highest on average without experiencing a significant decrease in grain protein content, although this increase was not statistically significant. [a] Heading date was determined by the julian date on which >50% of spikes 100% out of boot. Highest values in each column are underlined, and the highest significant values are bolded according to calculated LSD values.

Vida			Grain Yield	Maturity Date[a]	Tillers at Maturity	Productive Spikes	Height	Spikelets/ Spike	Grain Protein Content	Single Grain Weight
TB-A1	**TB-B1**	**TB-D1**	kg ha^{-1}	d	tillers m^{-2}	spikes m^{-2}	cm	no	g kg^{-1}	mg
wt	wt	wt (D1b)	**5024**	**233.0**	714	695	**84.7**	**13.3**	**146**	**29.0**
R256*	wt	wt (D1b)	**5293**	**233.0**	**827**	**801**	82.4	13.0	**145**	28.4
wt	wt	Q17*	**5158**	**233.0**	787	771	**84.9**	13.2	**146**	**29.6**
R256*	wt	Q17*	**5131**	**233.0**	**844**	**813**	82.8	**13.3**	**145**	28.0
wt	R127W	R125W	**5010**	232.0	**859**	**825**	82.3	**13.6**	143	27.7
R256*	R127W	R125W	4728	232.0	**875**	**829**	79.1	**13.8**	142	24.8
		LSD Value	365	0.79	57	53	1.07	0.45	1.9	0.69
		p from ANOVA								
		Genotype	0.020	0.008	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
		Environment	0.544	<0.001	0.003	0.002	0.233	0.014	0.841	<0.001
		GxE	0.977	0.154	0.174	0.170	<0.001	0.094	0.592	0.583

Figure 6.2. Duclair NIL radar plots showing mean proportions of measured yield components. Lines containing *TB-A1*-R256*, *TB-B1a*, and *TB-D1*-Q17* alleles (pink) yielded highest and showed a large increase in productive spike number without a decrease in spikelets/spike, or single grain weight relative to wildtype. Genotype labels are notated in order from A genome homoeolog to D genome homoeolog *(TB-A1 – TB-B1 – TB-D1)* labeled as either wildtype (WT) or respective *TB1* mutation.

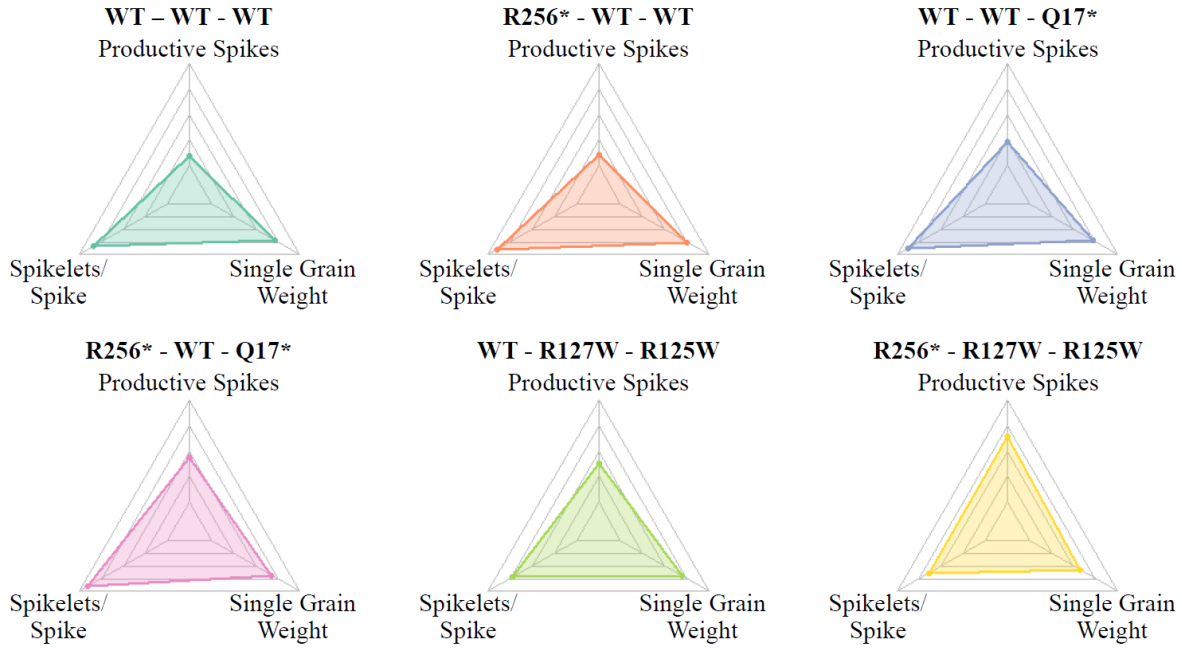


Figure 6.3. Duclair NIL bar plot showing how yield component proportions contribute to realized mean grain yield of each *TB1* genotype group. Lines containing *TB-A1*-R256*, *TB-B1a*, and *TB-D1*-Q17* alleles (pink) had the highest average grain yield, significantly higher than wildtype. *Significant at the .05 probability level.

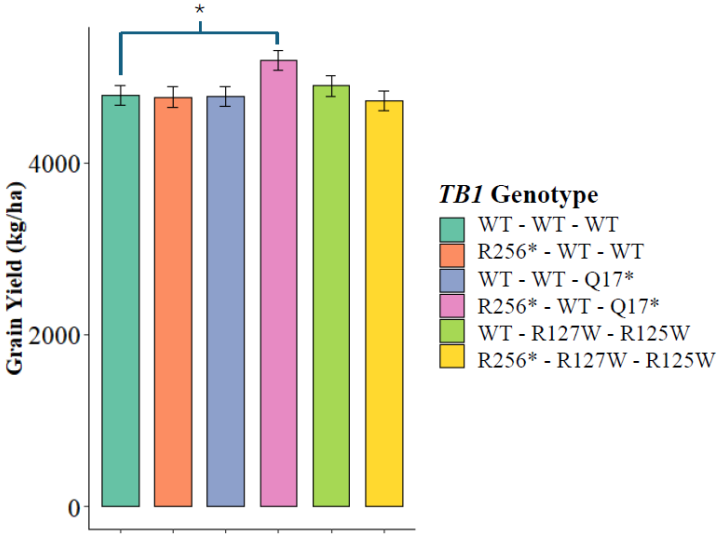


Figure 6.4. Vida NIL radar plots showing mean proportions of measured yield components. Lines containing *TB-A1*-R256*, *TB-B1a*, and *TB-D1a* alleles (orange) experienced an increase in productive spikes similar to other mutant genotypes without a decrease in spikelets per spike or single grain weight relative to wildtype.

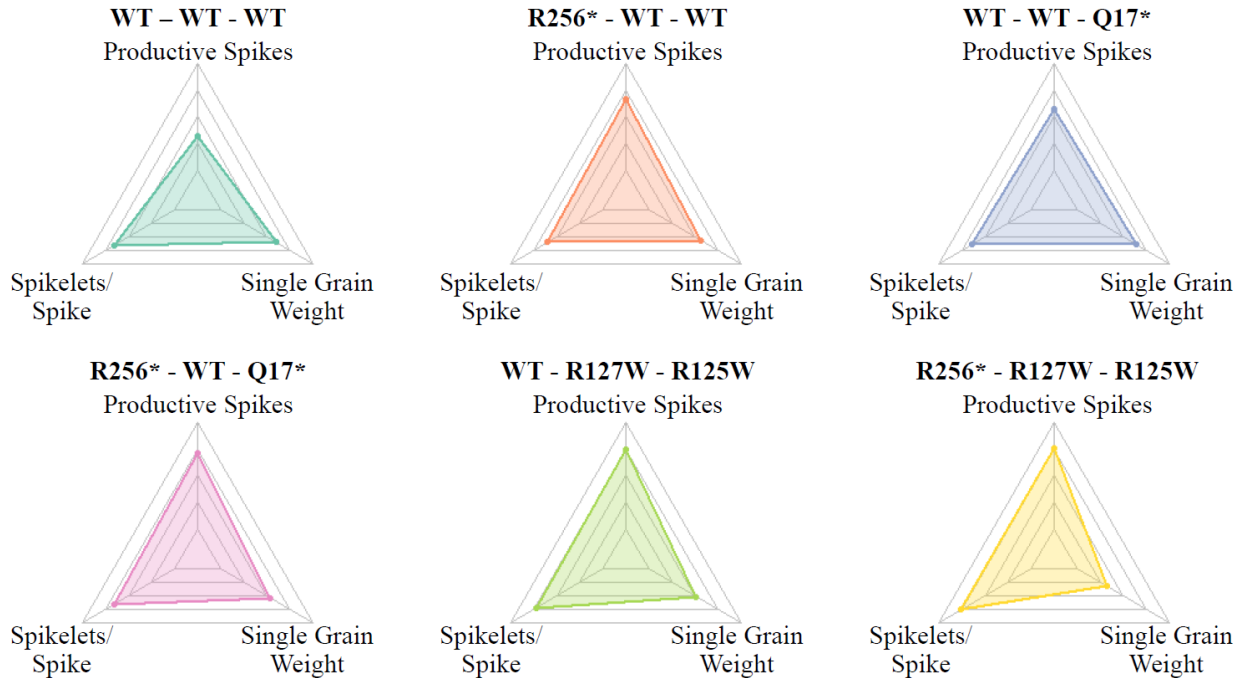
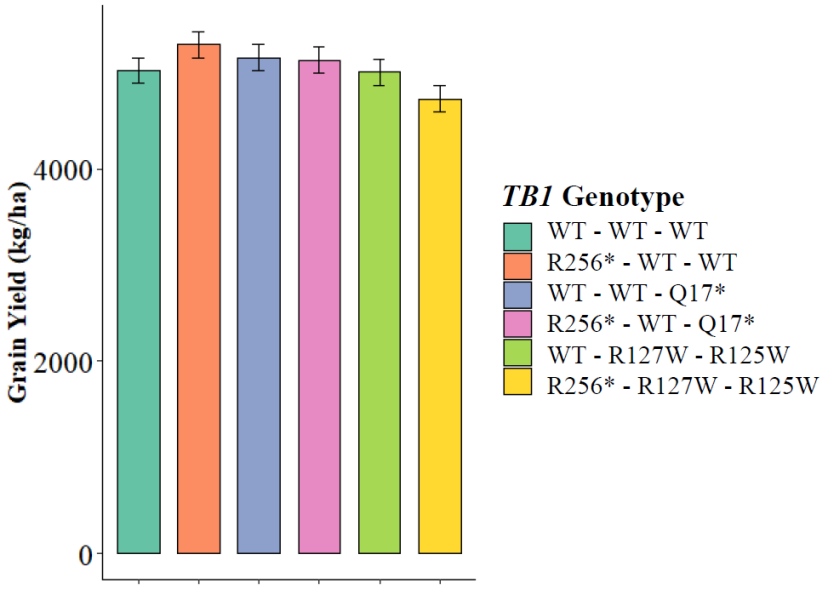


Figure 6.5. Vida NIL bar plot showing how yield component proportions contribute to realized grain yield of each *TB1* genotype group. Lines containing *TB-A1*-R256*, *TB-B1a*, and *TB-D1a* alleles (orange) showed the highest mean grain yield. No groups had significantly higher grain yield than wildtype.



Discussion

TB1 and *Rht-1* linkages

Differences in relative chromosomal location between *Rht-1* and *TB1* homoeologs is explained by the chromosome 4A pericentric inversion event first characterized in Devos et al. (1995). This inversion causes apparent differences in relative location of noted genes on the group four chromosomes, moving *Rht-A1* and *TB-A1* closer to the centromere of chromosome 4A. This creates tighter linkage between these genes relative to 4B and 4D homoeologs (Table 6.3).

Yield Increase From *TB-D1b* Natural Mutant

Although natural mutant alleles *TB-D1b* and *TB-B1b* are both predicted to be deleterious, neither of them have previously been documented to increase tiller number or improve yields. Although this study does not investigate *TB-B1b*, research shows that in Montana in a rainfed environment over two years, the *TB-D1b* allele conferred a significant yield advantage within the studied RIL population (Table 6.4). This result is indirectly supported by the findings of Dixon et al. (2018) which show a higher frequency of *TB-D1b* allele in wheat cultivars in the UK, France, and Belgium as compared to other regions, suggesting that this allele may confer an adaptive advantage in certain climates. No increase in tiller number was observed in *TB-D1b* mutant lines, which may have been a result of the relatively high seeding rate used in these experiments (297 seeds per m^2). Further experiments examining lines with induced mutations show that mutant alleles in combinations of two can provide a more pronounced yield increase than the natural *TB-D1b* mutation. Both *TB-D1b* and *TB-B1b* together may confer a significant tiller increase relative to *TB-D1b* alone, potentially resulting in the highest possible yield boost from natural TB1

mutant alleles. However, these alleles are not known to occur together in any cultivar. This may be because neither of these alleles are linked to either *Rht-B1b* or *Rht-D1b*, and are instead linked to the wildtype *Rht-1* alleles conferring a tall phenotype (an increasingly undesirable trait in modern varieties). One way to combine the two natural mutant *TB1* alleles in a semidwarf background would be to include an unlinked, non *Rht-1* allele that provides a semidwarfing phenotype, such as *Rht-8* or *Rht-13* (Gasperini et al., 2012; Rebetzke et al., 2011). Another method would be to artificially recreate the *TB-D1b* allele in a background already containing the *Rht-D1b* semidwarfing allele through EMS mutagenesis or CRISPR-Cas9 base pair editing techniques.

Yield Increases in Induced Mutation NIL

This study showed that yield increases resulting from introgression of EMS derived *TB1* mutations was dependent on genetic background. Vida background lines produce more tillers on average than Duclair background lines, and they also yielded more on average in this experiment (Table 6.5 and Table 6.6). Thus, Vida background lines would need a less pronounced productive tiller number increase than Duclair background lines to produce highest possible yields. These findings indicate that more tillers conferred by one or two *TB1* missense alleles increases grain yield, although the optimal dosage of these alleles is dependent on the tillering potential of the background prior to mutant allele introduction. This study supports the introduction of missense alleles *tb-A1*-R256* and *tb-D1*-Q49* in segregating breeding populations to create single and double *TB1* mutant lines that will increase tillering potential to varying selectable degrees.

Vida and Duclair both contain the natural mutant allele *TB-D1b.* Although no deliberate comparisons are made between this allele and EMS induced mutant alleles, comparisons can be

made between specific NIL genotype groups to infer their relative effects. Lines containing *tb-A1*-R256*, *TB-B1a*, and *TB-D1b* alleles can be compared with lines containing *tb-A1*-R256*, *TB-B1a,* and *tb-D1*-Q49* alleles to contrast the effects of *TB-D1b* with the effects of *tb-D1*-Q49*. Although there are no significant differences between these two genotype classes in the Vida background (Table 6.6), there are significant differences in the Duclair background (Table 6.5), with the *tb-A1*-R256*, *TB-B1a,* and *tb-D1*-Q49* genotype group conferring significantly more tillers and higher grain yield. This comparison indicates that the *TB-D1b* allele is not a full knockout of function allele since nonsense mutant alleles can confer a more significant effect depending on background. These inferences are consistent with Dixon et al. (2018) which showed through yeast two-hybrid analysis that the *TB-D1b* allele confers decreased binding between TB1 and a protein it regulates, FLOWERING LOCUS T1 (FT1), but not complete knock out of function. No direct unbiased comparisons can be made between the *tb-B1*-R127W and *tb-D1*-R125W missense mutation alleles and the *tb-A1*-R256* and *tb-D1*-Q49* nonsense mutation alleles to determine their relative effects. However, these missense alleles certainly confer phenotypic effects relative to wildtype in both backgrounds. Additionally, the novel multi row spikelet phenotype in *tb-A1*-R256*, *tb-B1*-R127W, and *tb-D1*-R125W triple mutant lines (Figure 6.1) not observed in any other genotype class, suggests that a genotypic threshold such as complete knockout of function is achieved in this group, although expression analysis using quantitative reverse transcriptase PCR or other methods are needed to confirm this hypothesis.

Increases in tillering are previously shown to increase yields in certain Montana environments (Jones et al., 2021; Nasseer et al., 2016). Increasing yields by increasing tiller number through introgression of *TB1* mutations is indirectly supported by two studies showing

decreases in *TB1* expression or function correlated with increased grain yields in stress inducing environments. Zhou et al. (2022) demonstrated increased drought and low nitrogen tolerance in wheat lines containing the overexpressed transgene *GmTDN1* from soybean (*Glycine max* L.). ChIP-seq transcriptome data (confirmed with qPCR analysis) showed that these lines had lower expression of *TB1* by a factor of approximately one-half. These transgenic lines showed increases in productive tiller number per m^2 and increases in grain yield in rainfed and lowly irrigated environments as well as in low nitrogen environments. In another study by Ishizaki et al. (2023) CRISPR-Cas9 induced missense mutations of Os*TB1* in Rice (*Oryza sativa* L.) enhanced tillering potential. These alleles conferred increased yields in low phosphorous greenhouse environments , but did not increase yields when grown in nutrient rich soil. Both studies were performed in warmer and wetter conditions relative to Montana. Together with the results of this study, these studies suggest that while increased tillering due to reduction of *TB1* function does not seem to increase yields in ideal growing conditions, grain yields are realized in environments stressed by either water or nutrient deficiencies.

TB1 Affects Plant Height

This study contrasts the findings of Dixon et al. (2020) which showed that the natural mutant allele *TB-D1b* confers an increase in plant height. Vida/Spring Yellowstone RIL population experiments found that lines containing the *TB-D1b* mutant allele trended towards being slightly shorter on average (between 0.5 and 0.9 cm shorter depending on the environment and year), although this decrease was not significant (Table 6.4). Additionally, experiments on induced mutant alleles show a highly significant ($p < 0.001$, one way ANOVA) dosage dependent decrease with the full wildtype genotype being the tallest, and mutant alleles of *TB1* conferring

progressively shorter plant height (Table 6.5 and Table 6.6). The decrease in plant height demonstrated in this study could be attributed to more resource allocation towards increases in yield and tiller number, and away from plant height. Experiments measuring height in Dixon et al. (2020) were conducted in a greenhouse, indicating a possible genotype by environment interaction. No genes that directly influence height have been found downstream of *TB1*.

Multi-row Spikelet Phenotype

Several types of abnormal wheat spike types containing supernumerary spikelets have previously been characterized. Normal wheat spikes typically have a single spikelet originating from each rachis node. Paired spikelets are a type of supernumerary spikelet occurring when two spikelets originating from a single rachis node are stacked vertically (Boden et al., 2015). When *TB1* is overexpressed, *FT1* (which induces flower development) is downregulated. A weak flowering signal resulting from *FT1* mutation or *TB1* overexpression results in paired spikelets (Dixon et al., 2018). Multi-row spikelets are visually different from paired spikelets and occur when two or more spikelets form horizontally (instead of vertically) from a single rachis node (Figure 6.1) (Dobrovolskaya et al., 2009; Dobrovolskaya, 2020). The multi-row spikelet phenotype observed in this study has similarities to the six-row barley (*Hordeum vulgare* ssp. *vulgare*) spike phenotype, although in barley, each spikelet contains one floret and thus one seed, unlike wheat spikelets which contain multiple florets and seeds. Barley row type is primarily controlled by the gene *Six-Rowed Spike 1 (VRS1).* The ancestral allele of this gene, *Vrs1.b*, confers a two-rowed spike phenotype, while a loss of function allele *vrs.1a* confers a six-rowed spike phenotype (Gauley & Boden, 2019; Komatsuda et al., 2007). These barley phenotypes can be modified through allelic variation in various ‘intermedium’ genes (Zwirek et al., 2019). One

of these genes called *Intermedium-C (INT-C)* or *Six-Rowed Spike 5 (VRS5)* is known to be an orthologue of *TB1* (Ramsay et al., 2011). The ancestral *INT-C* allele, *int-c.b*, partially represses lateral spikelet growth and occurs concurrently with the ancestral *VRS-1* allele *vrs1.a*, together conferring a two-row phenotype. Likewise, the loss of function allele, *int-c.a* occurs concurrently with loss of function *VRS1* allele *vrs.1a* together conferring a six-row phenotype. Mismatching these alleles in experimental NIL confers an intermediate barley spike phenotype, with three florets forming at some, but not all rachis nodes (Alqudah et al., 2016; Zwirek et al., 2019). Given that *TB1* and *INT-C* are orthologous, it makes sense that a phenotype similar to the intermediate spike phenotype of single *int-c.a* mutants in barley was observed in triple mutant *TB1* wheat spikes (Figure 6.1). The *Grain Number Increase 1 (GNI-1)* gene in wheat has been characterized as an orthologue of *VRS-1* in barley. Natural, loss of function alleles in *GNI-A1* confer an increase in the number of fertile florets per spikelet but not in a change in the actual number of spikelets originating from each rachis node (Golan et al., 2019; Hale et al., 2024; Sakuma et al., 2019). No full knockout lines of *GNI-1* have been characterized in wheat. To further investigate this novel multi-row spikelet phenotype, further research could be done in *GNI-1* to create full knockout lines to potentially be combined with triple mutant *TB1* lines to examine their combined effect on this multi-row spikelet phenotype.

Dobrovolskaya et al. (2015) characterized the gene *Wheat Frizzy Panicle (WFZP)*, an AP2/ERF (APETALA2/ethylene response factor) transcription factor that regulates inflorescence architecture. Mutant alleles of *WFZP* confer multi-row spikelet phenotype with branch-like multi spikelet structures forming at each node (Dobrovolskaya et al., 2015). Wang et al. (2022) further demonstrated multi-row spikelet phenotype in lines containing CRISPR-Cas9 generated mutant

alleles of a different AP2/ERF transcription factor gene, *DUO-B1*. These *DUO-B1* mutant lines had a multi-row spikelet phenotype identical to that of *TB1* triple mutant lines, with two to three spikelets forming directly next to each other at lower to middle rachis nodes. RNA seq experiments comparing *DUO-B1* mutant lines to wildtype showed a relative decrease in expression of both *WFZP* and *TB1*. It was postulated that the decrease in *WFZP* expression contributed to the formation of these multi-row spikelets (Sakuma & Koppolu, 2023; Wang et al., 2022). However, the spike phenotypes documented in Wang et al. (2022) match the phenotypes documented in this study more closely than the 'miracle wheat' phenotype documented in Dobrovolskaya et al. (2015). Results from this study show that the multi-row spikelet phenotype shown in Wang et al. (2022) may be a direct result of strong downregulation of *TB1* and consequent upregulation of *FT*, potentially inducing this novel spike phenotype.

Continued research

Additional field trials are being conducted to confirm the yield boosting effect of single and double *TB1* mutant NIL in Vida and Duclair backgrounds. Additional trials should also evaluate these lines under low-nitrogen and low-phosphorous conditions. This approach would mirror the experiments of Ishizaki et al. (2023) and Zhou et al. (2022) which demonstrated that increased tillering—at least partly due to reduced *TB1* function—can enhance grain yields in low nutrient environments. Studies should also examine the transcriptome of *TB1* triple mutant lines and compare with results from Wang et al. (2022) *DUO-B1* mutant studies. Differentially expressed genes that appear in both studies may help to elucidate which downstream genes are responsible for the observed multi-row spikelet phenotype.

Conclusions

Selecting for the naturally occurring *TB-D1b* mutant allele in segregating breeding populations to enrich for this beneficial allele could help to improve yield when breeding for a dryland environment. Additionally, introducing one or both EMS derived mutant alleles *tb-A1*-R256* and *tb-D1*-Q49* into Montana wheat breeding programs should increase yield by increasing tillering while maintaining ability to produce large spikes and high single grain weight. Although not agronomically beneficial, triple mutant *TB1* lines displayed an interesting multi row spikelet phenotype, demonstrating an additional way that *TB1* affects plant morphology.

Appendix Information

See Table D6.1 in Appendix D for Vida/Spring Yellowstone RIL population means split up by all environments and years of the study.

References

Alqudah, A. M., Koppolu, R., Wolde, G. M., Graner, A., & Schnurbusch, T. (2016). The Genetic Architecture of Barley Plant Stature. *Frontiers in Genetics*, *7*. https://doi.org/10.3389/fgene.2016.00117

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

Blake, N. K., Stougaard, R. N., Bohannon, B., Weaver, D. K., Heo, H. Y., Lamb, P. F.,…Talbert, L. E. (2014). Registration of 'Egan' Wheat with Resistance to Orange Wheat Blossom Midge. *Journal of Plant Registrations*, *8*(3), 298-302. https://doi.org/10.3198/jpr2014.04.0022crc

Boden, S. A., Cavanagh, C., Cullis, B. R., Ramm, K., Greenwood, J., Finnegan, E. J.,…Swain, S. M. (2015). Ppd-1 is a key regulator of inflorescence architecture and paired spikelet development in wheat. *Nature Plants*, *1*(2), 1-6. https://doi.org/10.1038/Nplants.2014.16

Bolser, D. M., Staines, D. M., Perry, E., & Kersey, P. J. (2017). Ensembl Plants: Integrating Tools for Visualizing, Mining, and Analyzing Plant Genomic Data. In (pp. 1-31). Springer New York. https://doi.org/10.1007/978-1-4939-6658-5_1

Bruckner, P. L., Berg, J. E., Riveland, N., Eckhoff, J. L., Wichman, D. M., Kephart, K. D.,…Chen, X. (2007). Registration of 'Yellowstone' Wheat. *Journal of Plant Registrations*, *1*(1), 18-19. https://doi.org/10.3198/jpr2006.12.0788crc

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

Cook, J. P., Heo, H. Y., Varella, A. C., Lanning, S. P., Blake, N. K., Sherman, J. D.,…Talbert, L. E. (2018). Evaluation of a QTL Mapping Population Composed of Hard Red Spring and Winter Wheat Alleles Using Various Marker Platforms. *Crop Science*, *58*(2), 701-712. https://doi.org/10.2135/cropsci2017.08.0488

Dixon, L. E., Greenwood, J. R., Bencivenga, S., Zhang, P., Cockram, J., Mellers, G.,…Boden, S. A. (2018). TEOSINTE BRANCHED1 Regulates Inflorescence Architecture and Development in Bread Wheat (Triticum aestivum). *Plant Cell*, *30*(3), 563-581. https://doi.org/10.1105/tpc.17.00961

Dixon, L. E., Pasquariello, M., & Boden, S. A. (2020). TEOSINTE BRANCHED1 regulates height and stem internode length in bread wheat. *Journal of Experimental Botany*, *71*(16), 4742-4750. https://doi.org/10.1093/jxb/eraa252

Dobrovolskaya, O., Martinek, P., Voylokov, A. V., Korzun, V., Roder, M. S., & Borner, A. (2009). Microsatellite mapping of genes that determine supernumerary spikelets in wheat (T. aestivum) and rye (S. cereale). *Theoretical and Applied Genetics*, *119*(5), 867-874. https://doi.org/10.1007/s00122-009-1095-1

Dobrovolskaya, O., Pont, C., Sibout, R., Martinek, P., Badaeva, E., Murat, F.,…Gautier, N. (2015). FRIZZY PANICLE drives supernumerary spikelets in bread wheat. *Plant Physiology*, *167*(1), 189-199. https://doi.org/10.1104/pp.114.250043

Dobrovolskaya, O. B. (2020). Supernumerary Spikelet Wheat Forms as Models for Studying Genetic Regulation of Inflorescence Development. *Russian Journal of Genetics*, *56*(11), 1298-1307. https://doi.org/10.1134/s1022795420110034

Doebley, J., Stec, A., & Gustus, C. (1995). Teosinte Branched1 and the Origin of Maize - Evidence for Epistasis and the Evolution of Dominance. *Genetics*, *141*(1), 333-346. https://doi.org/10.1093/genetics/141.1.333

Feiz, L., Martin, J., & Giroux, M. (2009). Creation and functional analysis of new Puroindoline alleles in Triticum aestivum. *Theoretical and Applied Genetics*, *118*(2), 247-257. https://doi.org/10.1007/s00122-008-0893-1

Gasperini, D., Greenland, A., Hedden, P., Dreos, R., Harwood, W., & Griffiths, S. (2012). Genetic and physiological analysis of Rht8 in bread wheat: an alternative source of semi-dwarfism with a reduced sensitivity to brassinosteroids. *Journal of experimental botany*, *63*(12), 4419. https://doi.org/10.1093/jxb/ers138

Gauley, A., & Boden, S. A. (2019). Genetic pathways controlling inflorescence architecture and development in wheat and barley. *Journal of Integrative Plant Biology*, *61*(3), 296-309. https://doi.org/10.1111/jipb.12732

Golan, G., Ayalon, I., Perry, A., Zimran, G., Ade-Ajayi, T., Mosquna, A.,…Peleg, Z. (2019). GNI-A1 mediates trade-off between grain number and grain weight in tetraploid wheat. *Theoretical and Applied Genetics*, *132*(8), 2353-2365. https://doi.org/10.1007/s00122-019-03358-5

Gutierrez-Gonzalez, J. J., Mascher, M., Poland, J., & Muehlbauer, G. J. (2019). Dense genotyping-by-sequencing linkage maps of two Synthetic W7984×Opata reference populations provide insights into wheat structural diversity. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-018-38111-3

Hale, C., Tillett, B., Martin, J., Hogg, A., Cook, J., & Giroux, M. (2024). The Grain Number Increase 1 alleles GNI-A1-105Y and-105K increase grain number in spring wheat. *Crop Science*. https://doi.org/10.1002/csc2.21267

Heo, H.-Y., Blake, N., Lanning, S., Lamb, P., Nash, D., Wichman, D.,…Reddy, G. (2018). Registration of 'NS Presser CLP'Hard Red Spring Wheat. *Journal of Plant Registrations*, *12*(1), 70-73. https://doi.org/10.3198/jpr2017.02.0008crc

Ishizaki, T., Ueda, Y., Takai, T., Maruyama, K., & Tsujimoto, Y. (2023). In-frame mutation in rice *TEOSINTE BRANCHED1 (OsTB1)* improves productivity under phosphorus deficiency. *Plant Science*, *330*, 111627. https://doi.org/10.1016/j.plantsci.2023.111627

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

Jones, B. H., Blake, N. K., Heo, H. Y., Martin, J. M., Torrion, J. A., & Talbert, L. E. (2021). Allelic response of yield component traits to resource availability in spring wheat. *Theoretical and Applied Genetics*, *134*(2), 603-620. https://doi.org/10.1007/s00122-020-03717-7

Komatsuda, T., Pourkheirandish, M., He, C., Azhaguvel, P., Kanamori, H., Perovic, D.,…Tagiri, A. (2007). Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. *Proceedings of the National Academy of Sciences*, *104*(4), 1424-1429. https://doi.org/10.1073/pnas.0608580104

Krasileva, K. V., Vasquez-Gross, H. A., Howell, T., Bailey, P., Paraiso, F., Clissold, L.,…Dubcovsky, J. (2017). Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(6), E913-E921. https://doi.org/10.1073/pnas.1619268114

Lanning, S., Carlson, G., Lamb, P., Nash, D., Wichman, D., Kephart, K.,…Eckhoff, J. (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., Carlson, G., Nash, D., Wichman, D., Kephart, K., Stougaard, R.,…Talbert, L. (2004). Registration of 'Choteau' wheat. *Crop science*, *44*(6), 2264-2266.

Lanning, S., Martin, J., Stougaard, R., Guillen-Portal, F., Blake, N., Sherman, J.,…Carlson, G. (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

Lanning, S., Talbert, L., McGuire, C., Bowman, H., Carlson, G., Jackson, G.,…Stallknecht, G. (1994). Registration of 'McNeal' wheat. https://doi.org/10.2135/cropsci1994.0011183X003400040060x

Lanning, S. P., Carlson, G. R., Nash, D., Wichman, D. M., Kephart, K. D., Stougaard, R. N.,…Talbert, L. E. (2006). Registration of 'Vida' wheat. *Crop Science*, *46*(5), 2315-2316. https://doi.org/10.2135/cropsci2006.03.0167

Lewis, J. M., Mackintosh, C. A., Shin, S., Gilding, E., Kravchenko, S., Baldridge, G.,…Muehlbauer, G. J. (2008). Overexpression of the maize Teosinte Branched1 gene in wheat suppresses tiller development. *Plant Cell Reports*, *27*(7), 1217-1225. https://doi.org/10.1007/s00299-008-0543-8

Li, X.-P., Lan, S.-Q., Liu, Y.-P., Gale, M., & Worland, T. (2006). Effects of different Rht-B1b, Rht-D1b and Rht-B1c dwarfing genes on agronomic characteristics in wheat. *Cereal research communications*, *34*(2-3), 919-924. https://doi.org/10.1556/crc.34.2006.2-3.220

NCEI (National Centers for Environmental Information). (2025). Daily summaries station details: Bozeman 6 W Experimental Farm, MT US, GHCND:USC00241047. Climate Data Online (CDO) | National Centers for Environmental Information (NCEI). https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00241047/detail

Naruoka, Y., Talbert, L. E., Lanning, S. P., Blake, N. K., Martin, J. M., & Sherman, J. D. (2011). Identification of quantitative trait loci for productive tiller number and its relationship to agronomic traits in spring wheat. *Theoretical and Applied Genetics*, *123*(6), 1043-1053. https://doi.org/10.1007/s00122-011-1646-0

Nasseer, A., Martin, J., Heo, H., Blake, N., Sherman, J., Pumphrey, M.,…Talbert, L. (2016). Impact of a quantitative trait locus for tiller number on plasticity of agronomic traits in spring wheat. *Crop Science*, *56*(2), 595-602. https://doi.org/10.2135/cropsci2015.05.0325

Ramsay, L., Comadran, J., Druka, A., Marshall, D. F., Thomas, W. T. B., Macaulay, M.,…Waugh, R. (2011). INTERMEDIUM-C, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene TEOSINTE BRANCHED 1. *Nature Genetics*, *43*(2), 169-U125. https://doi.org/10.1038/ng.745

Rasheed, A., Wen, W., Gao, F., Zhai, S., Jin, H., Liu, J.,…Xia, X. (2016). Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. *Theoretical and Applied Genetics*, *129*, 1843-1860. https://doi.org/10.1007/s00122-016-2743-x

Rebetzke, G., Ellis, M., Bonnett, D., Condon, A., Falk, D., & Richards, R. (2011). The Rht13 dwarfing gene reduces peduncle length and plant height to increase grain number and yield of wheat. *Field Crops Research*, *124*(3), 323-331. https://doi.org/10.1016/j.fcr.2011.06.022

Sakuma, S., Golan, G., Guo, Z. F., Ogawa, T., Tagiri, A., Sugimoto, K.,…Komatsuda, T. (2019). Unleashing floret fertility in wheat through the mutation of a homeobox gene. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(11), 5182-5187. https://doi.org/10.1073/pnas.1815465116

Sakuma, S., & Koppolu, R. (2023). Form follows function in Triticeae inflorescences. *Breeding Science*, *73*(1), 46-56. https://doi.org/10.1270/jsbbs.22085

U.S. Department of Agriculture (USDA). (2024). Small grains 2024 summary. U.S. Department of Agriculture, Economics, Statistics, and Marketing Information System. https://usda.library.cornell.edu/concern/publications/5t34sj573

Volkman, M., Martin, J., Hogg, A., Wright, L., Hale, C., Carr, P., & Giroux, M. (2022). Durum wheat Teosinte Branched1 null mutations increase tillering [Article]. *Crop Science*, *62*(4), 1522-1530. https://doi.org/10.1002/csc2.20775

Wang, M., Le Moigne, M.-A., Bertheloot, J., Crespel, L., Perez-Garcia, M.-D., Ogé, L.,…Sakr, S. (2019). BRANCHED1: A Key Hub of Shoot Branching. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.00076

Wang, Y., Du, F., Wang, J., Wang, K., Tian, C., Qi, X.,…Jiao, Y. (2022). Improving bread wheat yield through modulating an unselected AP2/ERF gene. *Nature Plants*, *8*(8), 930-939. https://doi.org/10.1038/s41477-022-01197-9

Zhou, Y., Liu, J., Guo, J., Wang, Y., Ji, H., Chu, X.,…Li, H. (2022). GmTDN1 improves wheat yields by inducing dual tolerance to both drought and low-N stress. *Plant Biotechnology Journal*, *20*(8), 1606-1621. https://doi.org/10.1111/pbi.13836

Zwirek, M., Waugh, R., & McKim, S. M. (2019). Interaction between row-type genes in barley controls meristem determinacy and reveals novel routes to improved grain. *New Phytol*, *221*(4), 1950-1965. https://doi.org/10.1111/nph.15548

CHAPTER SEVEN

KNOCKOUT MUTATIONS IN *TEOSINTE BRANCHED-1* A AND D GENOME HOMOEOLOGS INCREASE TILLERING IN FORAGE WINTER WHEAT

Abstract

Breeding forage winter wheat (*Triticum aestivum* L.) lines that can be harvested as whole plant biomass for animal feed has become more common in recent decades. Forage lines with higher tillering levels could potentially increase biomass and forage yield. Additionally, some systems allow for farmers to graze cattle on their winter wheat lines in the fall, while the plant is still in a vegetative state. Lines conferring an increase in early tillers would have improved fall forage biomass production in these systems. *Teosinte Branched-1 (TB1)* is a gene that controls branching in wheat and other plants. Decreases in *TB1* function increase tillering in wheat. Mutant *TB1* alleles *tb-A1*-R256* and *tb-D1*-Q49* were crossed into two Montana adapted forage winter wheat lines, 'Ray' and 'MTF20188'. Near isogenic lines (NIL) comparing different combinations of these alleles in the two backgrounds were developed, and these lines were tested in one year of field experiments. Results from this field study show that the introduction of these mutant alleles increases tiller number during milk stage, which is representative of a timepoint when these lines would be realistically harvested for forage. Future studies are planned to determine whether these tillering increases will increase forage yields in various systems.

Introduction

Other than its primary use as a grain crop, winter wheat (*Triticum aestivum* L.), there are several types of winter wheat forage systems. Winter wheat whole-plant biomass can serve as a forage crop, most commonly used to feed cattle. Winter wheat can also be grazed in the field during its vegetative state, and later harvested as a grain crop at maturity. Dual purpose winter wheat is a common component of cropping systems in the Southern Great Plains region of Texas, Oklahoma and surrounding states. This winter wheat is planted in the early fall, grazed by cattle during its vegetative state later that fall, and harvested at maturity the next summer as a standard grain crop. This management method is an important component of cropping systems in this region, since beef production and wheat grain production are central components of this region's agricultural economy. Successful implementation of this management practice results in maximum forage yields, and grain yield relies on optimization of planting dates, time periods of grazing, and variety selection (Edwards et al., 2011). Due to the importance of this practice in these systems, wheat breeders in this region will often integrate selection for varieties that produce high fall forage biomass as well as subsequent high grain yields into their programs (Carver et al., 2001).

In ranching systems, instead of being harvested for grain, certain winter wheat varieties can be harvested for forage prior to senescence between booting and hard dough stages (Zadoks growth stage 50 and growth stage 85) (Zadoks et al., 1974). Harvesting earlier is preferred when producing highly digestible feed suited for situations like feeding milking dairy cows, while later harvesting prioritizes higher forage yields when quality is less critical making it more suitable for situations like feeding beef cattle (Fohner, 2002; O'Keefe et al., 2022). Harvested forage winter

wheat can be stored directly after cutting in air tight silos or in wrapped plastic as silage, at a lower moisture content of between 50 and 60 % as haylage, or in dry bales with a moisture content of less than 20 % as hay (Cecava, 1995; Gordon et al., 1961). Harvesting wheat in any of these ways offers an early season source of single harvest forage before other forage crops like alfalfa (*Medicago sativa* L.) are harvestable. In warmer climates, forage winter wheat can be harvested early enough to facilitate double cropping systems (Fouli et al., 2012). Varieties planted for forage must be awnless or awnletted since awns or "beards" can be hazardous to cattle. Awns can get lodged in the mouths and throats of cattle, causing a serious fungal infection known as actinomycosis or "lump jaw" (Connaway & Uren, 1935). However, awns can be positively associated with grain yield, as they are photosynthetically active and provide assimilates directly to developing grains during grainfill (Ali et al., 2010). Thus, these forage varieties are generally special purpose and grown primarily for forage and not grain. 'Willow Creek' (PI 655073) is a commonly grown special purpose forage variety, released by the Montana Agricultural Experiment Station in 2005. This variety produces large yields of high quality forage. However, if harvested for seed or grain it produces low yields, and the end use quality of that grain is relatively low. More recently, Montana State University has released novel awnless winter wheat varieties 'MTF1435' (PI 689773) and 'Ray' (PI 689754) that can produce high yields of high quality forage and grain. (Bruckner et al., 2019). These varieties can be planted and later harvested either as a forage crop or as a grain crop depending on current market trends or due to any unplanned needs of the producer. Additionally, if these varieties are planted earlier in the fall in a system and climate that allows for it, they can be grazed during their vegetative states just like the dual purpose lines commonly grown in Texas and Oklahoma.

Consequently, these lines can be more than just dual purpose in the traditional sense. They are multipurpose in that they can be grazed in the fall, and later harvested as a cut forage or as a grain crop.

Increases in tiller number can be positively associated with increased biomass production and grain yield depending on environmental conditions (Sharma, 1993). Mutant alleles of *Teosinte Branched-1 (TB1)* homoeologs have been shown to increase tiller number in hexaploid wheat and in tetraploid durum wheat (*Triticum turgidum* L. subsp. *durum)* (Dixon et al., 2018; Volkman et al., 2022). *TB1* was first characterized in maize *(Zea mays* L. subsp. *mays)* as a key determinant between modern corn varieties and ancestral teosinte *(Zea mays* L. subsp. *parviglumis)*. It is a regulator of branching in plants and is part of the shade response pathway. Orthologues of *TB1* in dicot species are known as *Branched-1* or *BRC1*. Increases in *TB1* or *BRC1* expression in plants decreases branching, and decreases in expression increase branching (Aguilar-Martinez et al., 2007; Doebley et al., 1997; Wang et al., 2022; Whipple et al., 2011). Research in tetraploid durum wheat and in spring wheat has shown that *TB1* mutations increase grain yield in certain environments by increasing productive tiller number (Chapter 5 and Chapter 6). This same approach could be used to increase winter wheat forage yield.

Many studies have examined the relationships between grazing and harvested forage or grain yields. Studies show that with proper management, grazed winter wheat crops yield similarly, if not more than ungrazed crops. Grazing can reduce weed pressure and removes ancillary vegetative tissue which reduces early season water use and foliar disease pressure, potentially increasing grain yield in unfavorable conditions (Anderson & Impiglia, 2002; Edwards et al., 2011; Harrison et al., 2011). Conversely, if conditions are especially favorable,

grazing can reduce final plant height of the crop, reducing lodging and increasing harvestable yield (Redmon et al., 1995; Zeleke, 2019). One disproved traditional assumption is that grazing induces additional tillering in wheat through removal of the apical meristem, reducing apical dominance and enhancing tillering. Research has shown that grazing does not promote tillering, as during vegetative stages of development, vegetative tissue develops higher than actual apical meristems. Once apical meristems elongate upward and become accessible to grazing (around first hollow stem stage), reductions in harvested yield can occur if cattle remain in the field (Christiansen et al., 1989; Holman et al., 2009; Sharrow & Motazedian, 1987; Winter & Musick, 1991). While grazing does not enhance tillering, introduction of mutant *TB1* alleles is proven to do so. In fact, research shows that *TB1* mutations most reliably increase tiller number during the vegetative stage, which would increase grazeable fall biomass in these systems (Chapter 5).

Introgression of *TB1* mutations into dual purpose winter wheat has the potential to increase forage and grain yields in management systems with and without fall grazing. This increase in tiller number should be positively associated with fall vegetative biomass, harvestable forage biomass, and grain yield depending on conditions. In order to accomplish this goal, mutant alleles of *TB-A1* and *TB-D1* derived from the 'Cadenza' TILLING population were introgressed into two forage winter wheat lines, Ray and MTF20188 via backcrossing to create Near Isogenic Lines (NIL) (Krasileva et al., 2017). These NIL were grown in a small field experiment in Bozeman, MT in 2024, with additional plans to test them in future years and additional environments.

Materials and Methods

NIL Development

To create forage winter wheat NIL with varied decreases in *TB1* function, mutant alleles of *TB-A1* and *TB-D1* were combined. The *tb-A1*-R256* *(CGA to TGA)*, and *tb-D1*-Q49* *(CAA to TAA)* alleles were obtained from the Cadenza TILLING population (Krasileva et al., 2017). Cadenza is a European facultative winter wheat. These lines were crossed together to obtain a heterozygous *tb-A1*-R256* and *tb-D1*-Q49* mutant line. This line was crossed with Ray, a multipurpose winter wheat line containing the semidwarfing allele *Rht-B1b* (Bruckner et al., 2019; Pearce et al., 2011). Selected F_1 plants were backcrossed to Ray and were also crossed to the experimental winter wheat forage line MTF20188 which has a tall phenotype and does not contain any semidwarfing alleles. Ray and MTF20188 are both awnless, high tillering varieties with high forage yield and quality. Lines were backcrossed to their respective recurrent parents until the BC_5F_1 generation for the Ray background, and the BC_4F_1 generation for the MTF20188 background. At this point, the most vigorous F_1 plant heterozygous for both mutant alleles was selected for advancement to the F_2 generation. At each step, plants were screened to select for lines that were heterozygous for both mutant alleles. Genotyping was performed using allele specific qPCR PACE® genotyping markers (3CR Bioscience, UK) designed for the *tb-A1*-R256* and *tb-D1*-Q49* alleles (see chapter 5). Seedlings were vernalized in a vernalization chamber at four degrees Celsius for six to eight weeks. (Ray and MTF20188 lines require a minimum of six weeks of vernalization.)

In Spring 2023, 267 Ray background BC_5F_2 seeds and 254 MTF20188 background BC_4F_2 seeds were planted in small conetainers in a greenhouse and were genotyped for *tb-A1*-

R256* and *tb-D1*-Q49* alleles. Any lines heterozygous at either locus were discarded. In the MTF20188 background, 17 plants homozygous for the *tb-A1*-R256* and *tb-D1*-Q49* alleles, 17 plants homozygous for the *tb-A1*-R256* and *TB-D1a* alleles, 18 plants homozygous for the *TB-A1a* and *tb-D1*-Q49* alleles, and 16 plants homozygous for the *TB-A1a* and *TB-D1a* alleles were selected. In the Ray background, 20 plants homozygous for the *tb-A1*-R256* and *tb-D1*-Q49* alleles, 18 plants homozygous for the *tb-A1*-R256* and *TB-D1a* alleles, 13 plants homozygous for the *TB-A1a* and *tb-D1*-Q49* alleles, and 13 plants homozygous for the *TB-A1a* and *TB-D1a* alleles were selected. These selected plants were vernalized for eight weeks and then transplanted directly into the field at the Post Agronomy Farm in Bozeman, MT on 30 May 2023 with 15 cm spacing between plants. Plants were irrigated once on 17 July 2023 with approximately 5 cm of supplemental water by flood irrigation using a transportable water tank. No phenotypic parameters were measured in the field for these single plants. Plants were grown to maturity, harvested by hand, and threshed using a single plant thresher. Plants yielding less than 15 g of seed per plant were eliminated from the study. From the remaining lines, 10 NIL representing each of the eight genotype groups in the two different backgrounds were randomly selected for a total of 80 lines to be planted in the field in Fall 2023.

Experimental Design

The 80 selected NIL were planted at the Post Agronomy Farm in 3 m long, single row plots on 28 September 2023. These plots were planted at a density of seven grams of seed per row, equivalent to approximately 233 seeds per m^2. The experimental layout consisted of two separate but adjacent experiments for each background with two replicates each, organized in a randomized complete block design. Four respective parent check plots were included in each

replicate. Emergence and winter survival was uniform across all plots. However, certain plots experienced partial wash outs due to spring runoff, creating gaps within rows. Affected plots were excluded from relevant response variable analyses, such as tiller number and biomass yield.

Unfortunately, the *Rht-B1b* semidwarfing allele originating from the first cross between the Ray background line carrying the two mutant *TB1* alleles and MTF20188 had carried over into the BC_4F_1 MTF20188 plant selected to generate NIL. MTF20188 is a tall line carrying the *Rht-B1a* allele. Consequently, *Rht-B1a* and *Rht-B1b* segregated at an approximate ratio of 1:2:1 in single row BC5F2:3 plots grown in the field in 2024. Out of the 40 total BC5F2:3 lines planted, six NIL were fixed for *Rht-B1a*, including one *tb-A1*-R256* and *tb-D1*-Q49*, two *tb-A1*-R256* and *TB-D1a*, no *TB-A1a* and *tb-D1*-Q49*, and three *TB-A1a* and *TB-D1a*. Ten NIL were fixed for *Rht-B1b* including three lines fixed for *tb-A1*-R256* and *tb-D1*-Q49*, three *tb-A1*-R256* and *TB-D1a*, two *TB-A1a* and *tb-D1*-Q49*, and two *TB-A1a* and *TB-D1a*. The additional 24 MTF20188 background lines segregated for *Rht-B1a* and *Rht-B1b* alleles. All lines were included in the data analysis for the single row trial in 2024, but only tall lines fixed for *Rht-A1a* were selected to be planted in future trials.

Phenotyping

Heading date and maturity date were recorded for each plot. At milk stage (Zadok's stage of development 75), tiller number and leaf size were recorded. Tillers were counted within a 30 cm section of representative row within each plot. Three representative flag leaves were measured in each plot with length measured from the ligule to the tip of the leaf blade, and width measured at the widest point of the leaf blade. Forage samples were not collected, as grain yield was of primary importance to ensure adequate seed production to plant future trials. At maturity,

height was measured from ground level to the top of a representative spike, not including awns. Fertile spikelet number was recorded for three representative primary spikes within each plot. Primary spikes were defined as taller, larger spikes that were relatively more mature. Tiller number and productive spike number were recorded at maturity within a representative 30 cm section of row. Plots were determined to be mature when at least 50 % of peduncles had turned brown. Prior to harvest, lodging percentage was recorded for each plot. Due to severe lodging, each Ray background plot was cut by hand at ground level and bundled on 2 August 2024. Each MTF20188 background plot was cut and bundled using a Mitsubishi reaper binder (Mitsubishi Mahindra Agricultural Co., Ltd., Higashiizumo, Shimane, Japan) on 3 August 2024. Bundles were arranged in windrows to dry. All bundles were weighed and threshed using a Vogel threshing machine on 6 August 2024. Grain yield was measured prior to cleaning. Plot lengths were measured and equivalent grain yield and biomass yield was calculated.

Statistical analysis

Statistical Analysis

Analysis was performed using the lme4 package (Bates et al. 2015) in R (R Foundation for Statistical Computing. Version 4.0.5. Vienna, Austria). A mixed linear model was used to analyze the data recorded in this experiment, incorporating both fixed and random effects to account for genetic and experimental variation. Genotype was treated as a fixed effect, replication was included as a random effect to account for environmental variability across experimental replications, and each NIL line identifier was modeled as a random effect. The interaction between genotype and replicate was also included as a random effect to account for potential genotype-by-environment interactions between replications.

Results

Field Experiment Results

In field experiments, heading date and maturity date were uniform among the Ray background lines. Taller MTF20188 lines that were either fixed or heterozygous for *Rht-B1a* and *B1b* matured uniformly, while shorter MTF20188 lines fixed for *Rht-B1b* matured two days later (data not shown). All Ray and MTF20188 background lines were uniformly awnletted. MTF20188 NIL were all analyzed together, despite *Rht-B1* alleles randomly segregating within this population. Thus, variance for certain parameters such as height are much more variable in the MTF20188 background than in the Ray background (Table 7.1 and Table 7.2). Ray lines experienced more lodging than MTF20188 lines, however within backgrounds, lodging was random and was not associated with any genotype group (data not shown).

In the MTF20188 background NIL, tiller number at milk stage and at maturity trended up with the inclusion of *TB1* mutant alleles. The double mutant *tb-A1*-R256* and *tb-D1*-Q49* genotype produced the most tillers and productive spikes at each stage, and both single mutant genotypes tillered more than wildtype. Tillering differences were only statistically significant when comparing the double mutant genotype with wildtype at maturity (Table 7.1). In Ray background NIL, the single mutant genotypes tillered significantly more than the wildtype, while the double mutant lines tillered more than wildtype but slightly less than single mutant genotypes (Table 7.2). At maturity, double mutant genotypes had the most tillers and productive spikes on average overall, but this difference was not statistically significant. These differences in tiller number did not translate to differences in grain yield or in harvested biomass. In the Ray background, double mutant and single *tb-A1*-R256* mutant genotypes were shorter than

wildtype and single *tb-D1*-Q49* mutant genotypes (Table 7.2). In the MTF20188 background, double mutant genotypes had the shortest flag leaves on average (Table 7.1).

Table 7.1. MTF20188 background NIL genotype group averages. There were significant differences between genotypes in tiller number and productive spike number at maturity, spikelets per spike, and leaf length, but not in any other measured parameters. Values represent the average for each genotype ± the standard error. Means followed by different letters indicate they differ significantly ($P < .05$). T comparisons were made only following a significant ($P < .05$) F ratio.

TB1 Genotype		**Tillers at Milk Stage**	**Tillers at Maturity**	**Productive Spikes**
A allele	D allele	—— no. m^{-2} ——		
TB-A1	*TB-D1*	811±54	745±22 a	714±20 a
tb-A1-R256*	*TB-D1a*	899±54	789±22 ab	729±20 ab
TB-A1	*tb-D1*-Q17*	846±54	785±22 ab	744±21 ab
tb-A1-R256*	*tb-D1*-Q17*	935±53	859±21 b	805±20 b
	genotype *p* value	0.09	0.005	0.02

TB1 Genotype		**Spikelets per Spike**	**Grain Yield**	**Biomass**
A allele	D allele	no.	kg ha^{-1}	kg m^{-2}
TB-A1	*TB-D1*	19.0±0.2 ab	7909±225	8.86±0.35
tb-A1-R256*	*TB-D1a*	18.6±0.2 a	7721±231	8.18±0.35
TB-A1	*tb-D1*-Q17*	19.2±0.2 ab	8035±230	8.68±0.35
tb-A1-R256*	*tb-D1*-Q17*	19.5±0.2 b	7595±218	8.07±0.34
	genotype *p* value	0.0003	0.73	0.18

TB1 Genotype		**Height**	**Leaf Width**	**Leaf Length**
A allele	D allele	—— cm ——		
TB-A1	*TB-D1*	137±4.4	1.64±0.02	21.8±0.4 ab
tb-A1-R256*	*TB-D1a*	132±4.4	1.64±0.02	21.9±0.4 a
TB-A1	*tb-D1*-Q17*	136±4.4	1.62±0.02	20.9±0.4 ab
tb-A1-R256*	*tb-D1*-Q17*	132±4.4	1.59±0.02	20.2±0.4 b
	genotype *p* value	0.69	0.50	0.03

Table 7.2. Ray background NIL genotype group averages. There were significant differences between genotypes in tiller number at milk stage and in plant height, but not in any other measured parameters. Values represent the average for each genotype ± the standard error. Means followed by different letters indicate they differ significantly (P < .05). T comparisons were made only following a significant (P < .05) F ratio.

TB1 Genotype		**Tillers at Milk Stage**	**Tillers at Maturity**	**Productive Spikes**
A allele	D allele		no. m^{-2}	
TB-A1	*TB-D1*	792±35 a	747±40	665±24
tb-A1-R256*	*TB-D1a*	933±35 b	792±40	691±25
TB-A1	*tb-D1*-Q17*	913±36 b	755±42	646±27
tb-A1-R256*	*tb-D1*-Q17*	893±35 ab	811±40	715±24
	genotype *p* value	0.01	0.50	0.33

TB1 Genotype		**Spikelets per Spike**	**Grain Yield**	**Biomass Yield**
A allele	D allele	no.	kg ha^{-1}	kg m^{-2}
TB-A1	*TB-D1*	22.4±0.32	9290±286	2.44±0.065
tb-A1-R256*	*TB-D1a*	22.0±0.32	8788±286	2.34±0.065
TB-A1	*tb-D1*-Q17*	22.5±0.32	9227±302	2.5±0.069
tb-A1-R256*	*tb-D1*-Q17*	21.9±0.32	8788±286	2.35±0.065
	genotype *p* value	0.32	0.34	0.22

TB1 Genotype		**Height**	**Leaf Width**	**Leaf Length**
A allele	D allele		cm	
TB-A1	*TB-D1*	120±0.7 a	1.93±0.02	21.8±0.3
tb-A1-R256*	*TB-D1a*	117±0.7 b	1.86±0.02	21.3±0.3
TB-A1	*tb-D1*-Q17*	120±0.7 a	1.90±0.02	22.4±0.3
tb-A1-R256*	*tb-D1*-Q17*	118±0.7 ab	1.89±0.02	21.2±0.3
	genotype *p* value	0.05	0.17	0.10

Discussion

Height differences in Ray background NIL are similar to height differences seen in durum wheat and spring wheat *TB1* experiments, with single mutant *tb-D1* genotypes and having similar height to wildtype, and single *tb-A1* mutant and double mutant genotypes both having shorter plant height (Table 7.2, Chapter 5, and Chapter 6). Larger differences in tiller number at milk stage between *TB1* genotypes is consistent with *TB1* durum wheat experiments showing that tiller number differences between *TB1* genotypes at earlier stages of development can be greater than tiller number at maturity depending on environmental conditions (Chapter 5). Harvested biomass in this experiment was only measured at maturity. Considering greater differences in tiller number at milk stage, there may have been differences in biomass if forage samples had been harvested at a realistic timepoint. Forage biomass harvested during milk or soft dough stage will be measured in all future experiments to determine whether increases in tiller number will improve this parameter. Slightly smaller flag leaf size may suggest a lower leaf to stem ratio, which is an indicator of lower forage digestibility (Table 7.1 and Table 7.2) (Sinclair & No'am G, 1995). However, increases in tiller number may also be associated with a decrease in single stem mass, resulting in a net neutral change in leaf:stem ratios. Forage quality parameters including total digestible nutrients will be measured in future studies to determine if increases in tillering influence forage quality.

Planned Experiments for 2024-2025

In Fall 2024, multiple experiments were planted to continue testing *TB1* mutant NIL. Instead of planting individually derived NIL in each plot, NIL samples were combined in bulked composite entries representing multiple BC_5F_2 or BC_4F_2 derived NIL from each genotype group.

Because of the *Rht-B1* alleles segregating in the BC_5F_3 MTF20188 background field experiment, only one to three different NIL represented the composite entry for each genotype group in this background (Table 7.3). This excluded the *tb-D1*-Q49* single mutant genotype since no fixed *Rht-B1a* "tall" NIL from this group were generated. For Ray background samples, four random NIL from each genotype group were selected to be combined as composite entries (Table 7.3).

Table 7.3. Lines Used to Make Composite Entries for 2024-2025 Experiments. MTF20188 background line seed is currently at the $BC_4F_{2:4}$ generation, and Ray background line seed is currently at the $BC_5F_{2:4}$ generation.

2024 Plot Source	**Background**	**NIL ID**	***TB-A1* Genotype**	***TB-D1* Genotype**
130	MTF20188	TB1.MTF20188.353	*tb-A1*-R256*	*tb-D1*-Q49*
114	MTF20188	TB1.MTF20188.393	*tb-A1*-R256*	*TB-D1a*
109	MTF20188	TB1.MTF20188.406	*tb-A1*-R256*	*TB-D1a*
105	MTF20188	TB1.MTF20188.311	*TB-A1a*	*TB-D1a*
132	MTF20188	TB1.MTF20188.363	*TB-A1a*	*TB-D1a*
112	MTF20188	TB1.MTF20188.448	*TB-A1a*	*TB-D1a*
303	Ray	TB1.Ray.36	*tb-A1*-R256*	*tb-D1*-Q49*
304	Ray	TB1.Ray.5	*tb-A1*-R256*	*tb-D1*-Q49*
306	Ray	TB1.Ray.149	*tb-A1*-R256*	*tb-D1*-Q49*
328	Ray	TB1.Ray.117	*tb-A1*-R256*	*tb-D1*-Q49*
312	Ray	TB1.Ray.82	*tb-A1*-R256*	*TB-D1a*
327	Ray	TB1.Ray.64	*tb-A1*-R256*	*TB-D1a*
335	Ray	TB1.Ray.258	*tb-A1*-R256*	*TB-D1a*
340	Ray	TB1.Ray.80	*tb-A1*-R256*	*TB-D1a*
301	Ray	TB1.Ray.102	*TB-A1a*	*tb-D1*-Q49*
309	Ray	TB1.Ray.212	*TB-A1a*	*tb-D1*-Q49*
325	Ray	TB1.Ray.32	*TB-A1a*	*tb-D1*-Q49*
326	Ray	TB1.Ray.73	*TB-A1a*	*tb-D1*-Q49*
308	Ray	TB1.Ray.163	*TB-A1a*	*TB-D1a*
311	Ray	TB1.Ray.252	*TB-A1a*	*TB-D1a*
314	Ray	TB1.Ray.65	*TB-A1a*	*TB-D1a*
331	Ray	TB1.Ray.53	*TB-A1a*	*TB-D1a*

Experiments were planted to test Ray and MTF20188 background lines at two different seeding rates (standard and half standard seeding rate) with the hypothesis that mutant *TB1* lines would tiller much more than wildtype lines when planted at half density to recover standard seeding rate forage production levels. This would allow farmers to plant these lines at lower seeding rates. Additionally, this would demonstrate that TB1 mutant lines may show improved recovery from winter kill, as the increased tillering capacity of surviving plants would make up for killed neighboring plants. These experiments were planted in Bozeman, MT at the Post Agronomy Farm and in Havre, MT at the Northern Agricultural Research Center.

Composite NIL lines in Ray and MTF20188 backgrounds were tested at two different densities. These trials were designed as four separate, but adjacent experiments split by background and seeding rate. Standard seeding rate experiments in Havre were planted a rate of 233 seeds per m^2 and half density experiments were planted at 117 seeds per m^2. In Bozeman, experiments were planted at seeding rates of 210 seeds per m^2 and 105 seeds per m^2 respectively. Each experiment was set up as a balanced completely randomized design including six replicates of each composite NIL entry. Each experiment contained between two and six parent line checks depending on field dimensions. Havre experiments were planted in late September 2024, and Bozeman experiments were planted 15 October 2024. In both locations, plots consisted of three, five meter long rows spaced 30 cm apart.

Composite Ray background NIL were also sent to Agrifor Seeds LLC (Mendon, UT, USA) and South Dakota State University (SDSU) for inclusion in their respective forage winter wheat trials. For these two trials, registered seed of Ray was sent in place of the wildtype *TB1* NIL composite line that was used in Bozeman and Havre experiments to test *TB1* mutant NIL

genotypes against their respective parent lines in a balanced comparison to see whether backcrossed lines could be considered for re-release as new varieties. Five-pound samples of each composite entry were sent to Agrifor and to SDSU. Agrifor trials were planted in three different locations, and SDSU trials were planted in two different locations. These trials were all planted in late September, 2024. There are also plans to include *TB1* mutant Ray background NIL in 2025-2026 Kansas State University dual purpose winter wheat trials to test how these lines would perform when harvested for grain in a system with simulated fall grazing.

Conclusion

Introgressing *tb-A1*-R256* and *tb-D1*-Q49* mutant alleles into forage winter wheat lines Ray and MTF20188 increases tiller number at milk stage, which is a representative timepoint at which these lines would be harvested for forage. Future studies in Bozeman, MT; Havre, MT; South Dakota; Utah; and Kansas under various management conditions will show how this tiller difference affects forage yields and quality, as well as grain yield and quality.

References

Aguilar-Martinez, J. A., Poza-Carrion, C., & Cubas, P. (2007). Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. *Plant Cell*, *19*(2), 458-472. https://doi.org/10.1105/tpc.106.048934

Ali, M. A., Hussain, M., Khan, M. I., Ali, Z., Zulkiffal, M., Anwar, J.,…Zeeshan, M. (2010). Source-Sink Relationship between Photosynthetic Organs and Grain Yield Attributes during Grain Filling Stage in Spring Wheat (*Triticum aestivum* L.). *International Journal of Agriculture and Biology*, *12*(4), 509-515.

Anderson, W., & Impiglia, A. (2002). Management of dryland wheat. *Wheat: Improvement and Production. Plant Production and Protection Series*, *30*, 567.

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

Bruckner, P. L., Berg, J. E., Lamb, P. F., Carr, P., Wichman, D. M., Miller, J. H.,…Chen, X. (2019). Registration of 'Ray' Hard Red Winter Wheat. *Journal of Plant Registrations*, *13*(3), 392-395. https://doi.org/10.3198/jpr2019.03.0012crc

Carver, B., Khalil, I., Krenzer, E., & MacKown, C. (2001). Breeding winter wheat for a dual-purpose management system. *Euphytica*, *119*, 231-234. https://doi.org/10.1023/A:1017543800311

Cecava, M. J. (1995). Making hay and haylage. In *Beef cattle feeding and nutrition* (pp. 104-116). Elsevier. https://doi.org/10.1016/B978-012552052-2/50011-1

Christiansen, S., Svejcar, T., & Phillips, W. (1989). Spring and fall cattle grazing effects on components and total grain yield of winter wheat. *Agronomy Journal*, *81*(2), 145-150. https://doi.org/10.2134/agronj1989.00021962008100020002x

Connaway, J. W., & Uren, A. (1935). *Actinomycosis in cattle: lump jaw, big jaw, and wooden tongue*. University of Missouri, College of Agriculture, Agricultural Experiment Station.

Dixon, L. E., Greenwood, J. R., Bencivenga, S., Zhang, P., Cockram, J., Mellers, G.,…Boden, S. A. (2018). TEOSINTE BRANCHED1 Regulates Inflorescence Architecture and Development in Bread Wheat (Triticum aestivum). *Plant Cell*, *30*(3), 563-581. https://doi.org/10.1105/tpc.17.00961

Doebley, J., Stec, A., & Hubbard, L. (1997). The evolution of apical dominance in maize. *Nature*, *386*(6624), 485-488. https://doi.org/10.1038/386485a0

Edwards, J., Carver, B., Horn, G., & Payton, M. (2011). Impact of dual-purpose management on wheat grain yield. *Crop Science*, *51*(5), 2181-2185. https://doi.org/10.2135/cropsci2011.01.0043

Fohner, G. (2002, December). Harvesting maximum value from small grain cereal forages. In *Proceedings of the 32nd California alfalfa and forage symposium, Reno, Nevada'.(Department of Agronomy and Range Science Extension, University of California: Davis) Available at http://alfalfa.ucdavis.edu/*

Fouli, Y., Duiker, S., Fritton, D., Hall, M., Watson, J., & Johnson, D. (2012). Double cropping effects on forage yield and the field water balance. *Agricultural Water Management*, *115*, 104-117. https://doi.org/10.1016/j.agwat.2012.08.014

Gordon, C., Derbyshire, J., Wiseman, H., Kane, E., & Melin, C. (1961). Preservation and feeding value of alfalfa stored as hay, haylage, and direct-cut silage. *Journal of Dairy Science*, *44*(7), 1299-1311. https://doi.org/10.3168/jds.S0022-0302(61)89881-0

Harrison, M. T., Evans, J. R., Dove, H., & Moore, A. D. (2011). Dual-purpose cereals: can the relative influences of management and environment on crop recovery and grain yield be dissected? *Crop and Pasture Science*, *62*(11), 930-946. https://doi.org/10.1071/CP11066

Holman, J. D., Thompson, C. R., Hale, R. L., & Schlegel, A. J. (2009). Grazing effects on yield and quality of hard red and hard white winter wheat. *Agronomy Journal*, *101*(4), 775-788. https://doi.org/10.2134/agronj2008.0163x

Krasileva, K. V., Vasquez-Gross, H. A., Howell, T., Bailey, P., Paraiso, F., Clissold, L.,…Dubcovsky, J. (2017). Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(6), E913-E921. https://doi.org/10.1073/pnas.1619268114

O'Keefe, C., Penner, G., McKinnon, J., Larson, K., Damiran, D., & Lardner, H. B. (2022). Effects of stage of maturity at harvest of cereal crops on biomass and quality, estimated forage dry matter intake, beef cow performance, and system economics. *Applied Animal Science*, *38*(6), 607-619. https://doi.org/10.15232/aas.2022-02327

Pearce, S., Saville, R., Vaughan, S. P., Chandler, P. M., Wilhelm, E. P., Sparks, C. A.,…Phillips, A. L. (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

Redmon, L. A., Horn, G. W., Krenzer Jr, E. G., & Bernardo, D. J. (1995). A review of livestock grazing and wheat grain yield: Boom or bust? *Agronomy Journal*, *87*(2), 137-147. https://doi.org/10.2134/agronj1995.00021962008700020001x

Sharma, R. (1993). Selection for biomass yield in wheat. *Euphytica*, *70*, 35-42. https://doi.org/10.1007/BF00029638

Sharrow, S., & Motazedian, I. (1987). Spring Grazing Effects on Components of Winter Wheat Yield 1. *Agronomy Journal*, *79*(3), 502-504. https://doi.org/10.2134/agronj1987.00021962007900030019x

Sinclair, T. R., & No'am G, S. (1995). Global environment change and simulated forage quality of wheat I. Nonstressed conditions. *Field Crops Research*, *40*(1), 19-27. https://doi.org/10.1016/0378-4290(94)00091-P

Volkman, M. M., Martin, J. M., Hogg, A. C., Wright, L., Hale, C., Carr, P. M., & Giroux, M. J. (2022). Durum wheat Teosinte Branched1 null mutations increase tillering. *Crop Science*, *62*(4), 1522-1530. https://doi.org/10.1002/csc2.20775

Wang, H. W., Seiler, C., Sreenivasulu, N., Wiren, V., & Kuhlmann, M. (2022). INTERMEDIUM-C mediates the shade-induced bud growth arrest in barley. *Journal of Experimental Botany*, *73*(7), 1963-1977. https://doi.org/10.1093/jxb/erab542

Whipple, C. J., Kebrom, T. H., Weber, A. L., Yang, F., Hall, D., Meeley, R.,…Jackson, D. P. (2011). grassy tillers1 promotes apical dominance in maize and responds to shade signals in the grasses. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(33), E506-E512. https://doi.org/10.1073/pnas.1102819108

Winter, S., & Musick, J. (1991). Grazed wheat grain yield relationships. *Agronomy Journal*, *83*(1), 130-135. https://doi.org/10.2134/agronj1991.00021962008300010030x

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed research*, *14*(6), 415-421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zeleke, K. T. (2019). Effect of grazing time and intensity on growth and yield of spring wheat (Triticum aestivum L.). *Journal of Integrative Agriculture*, *18*(5), 1138-1147. https://doi.org/10.1016/S2095-3119(18)62125-2

CHAPTER EIGHT

CONCLUSION

Studies in this thesis contribute directly to variety development in Montana, providing breeding programs with new information and novel allelic diversity to manipulate seed size, plant height, and productive tiller number. Natural mutant alleles *GNI-A1*-105K and *GNI-A1*-105Y of *Grain Number Increase 1 (GNI-1)* increase grain number per spike and decrease seed size in spring wheat (*Triticum aestivum* L.) without affecting grain quality and milling traits (Chapter 2). This enables wheat breeders to use markers to select for larger or smaller seeds, without affecting other traits. The durum wheat *(Triticum turgidum* L. subsp. *durum)* ethyl methanesulfonate (EMS) derived allele *Rht-B1b*-E529K of *Reduced height-1 (Rht-1)* imparts an intermediate height phenotype between that of traditional semidwarf and tall varieties (Chapter 3). *Rht-B1b*-E529K improves seedling emergence, grain protein content, and grain yield compared to a semidwarf phenotype, and should improve lodging resistance compared to a tall phenotype. Similarly, the spring wheat EMS derived allele *Rht-A1*-E63K imparts an intermediate height phenotype and increases grain yield compared to a tall phenotype without decreasing grain protein content (Chapter 4). Mutant alleles of *Teosinte Branched 1 (TB1)* increase productive tiller number in durum, spring, and winter wheat. Eleven location/years of field experiments show that the EMS derived mutant durum wheat allele *tb-B1*-W341* increases tillering plasticity, increasing productive tiller number and grain yield in certain environments (Chapter 5). In spring wheat, the naturally occurring *TB-D1b* mutant allele improves yield in RIL population experiments in rainfed environments (Chapter 6). Additionally, EMS derived mutant spring wheat alleles *tb-A1*-R256* and *tb-D1*-Q49* improve spring wheat grain yields in two

different NIL population experiments (Chapter 6). Alleles *tb-A1*-R256* and *tb-D1*-Q49* also improve tillering in forage winter wheat in data from two different NIL population experiments (Chapter 7). Similar projects have been initiated in forage triticale *(Triticosecale spp.)* to improve tillering and biomass yields (Appendix E). These studies all provide wheat breeders with genetic tools and information to enhance yield and quality in their programs.

Future Research

Continued field trials of NIL varying for *TB1* alleles in spring wheat and in winter wheat will be grown in multiple locations in 2025 and 2026 to provide more conclusive evidence supporting their benefits. These trials will provide data needed for publication of these two studies. Beyond further experiments on developed NIL populations, studies in this thesis have informed the initiation of other studies that examine *GNI-A1, Rht-1,* and *TB1* in other ways.

The *GNI-A1* study has led to the initiation of a similar project in durum wheat. NIL populations have been developed to test how *GNI-A1* 105N and 105Y alleles affect seed size, grain yield, and cereal quality traits in Montana adapted durum varieties. The *GNI-A1* 105N allele is rare in North American durum germplasm. In spring wheat, *GNI-A1* 105N can confer larger seed size. Similar results in a durum wheat experiment may support enrichment for this allele in durum breeding programs to increase seed size through marker assisted selection.

Mutant *TB1* alleles can impart increases in grain yield and protein content beyond what can be explained by increases in tiller number and spike morphology, pointing to potential below ground effects. Experiments are being conducted in durum wheat examining differences in root morphology between NIL varying for *TB1* alleles. Reduced function *TB1* alleles are hypothesized to confer increased root branching and biomass, increasing resource uptake.

CRISPR-Cas9 generated edits and knockouts are being generated to further study *Rht-1* in durum, and to study *TB1* in canola (*Brassica napus* L.), and soybean (*Glycine max* L.). Artificial *Rht-A1b* alleles are being created in durum wheat, replicating sequences of *Rht-B1b* and *Rht-D1b* natural semidwarfing alleles in the A genome. Additionally, *Rht-B1b* alleles are being created in a durum wheat background, producing a B genome durum semidwarfing allele not linked to any residual hexaploid wheat genetic material originating from the transfer of *Rht-B1b* into durum wheat that may be causing linkage drag. Knockout alleles of *TB1* orthologs in soybean and canola are being created with the hypothesis that incorporating one knockout allele into each of these tetraploid crop species will create reduced function *TB1* genotypes with increased branching and productivity (Hu et al., 2022; Shim et al., 2019; Zhang et al., 2025). Transformed durum wheat and canola lines carrying edits and knockouts are currently being grown at the UC Davis plant transformation facility, with plans to send the resulting seed to MSU in 2025. Collaborations to develop *TB1* knockouts in soybean are currently being initiated.

Integration of Beneficial Alleles

Breeding populations containing alleles with demonstrated benefits are being developed to begin integration of beneficial alleles into Montana breeding programs. Lines containing the *Rht-B1b*-E529K, *tb-A1*-W339* and *tb-B1*-W341* alleles were incorporated into durum wheat crossing blocks in 2023. Resulting breeding populations were advanced and will be grown in head rows in 2025. Lines will be visually selected from these head rows for advancement to preliminary yield trials in 2026. If the proven benefits of these alleles hold up through future years of unbiased selection and trials, elite durum lines containing one or more of these alleles may be selected for release as a future variety. In spring wheat, crosses were made between

Duclair background NIL fixed for *tb-A1*-R256* and *tb-D1*-Q49* alleles, and Vida background NIL fixed for the same alleles to create spring wheat breeding populations with a fixed *TB1* mutant genotype. Similarly, crosses were made between Ray and MTF20188 background NIL containing these alleles to create fixed *TB1* mutant breeding populations in forage winter wheat. These populations are being advanced, and resulting F_5 derived lines will be evaluated to find potential candidate lines to release as new, high tillering varieties, or to use as genetic stock to introduce these beneficial alleles into additional populations. Similar plans are also in place for lines generated from integration of *TB1* alleles into triticale.

High tillering, intermediate height, elite durum lines created through backcrossing of *Rht-B1b*-E529K, *tb-A1*-W339* and *tb-B1*-W341* alleles into 'MT Raska' have been developed for potential release as an enhanced versions of this variety. Because *Rht-1* and *TB1* are closely linked, *Rht-B1b*-E529K and *tb-B1*-W341* cannot realistically be combined. Therefore, two separate schemes to create enhanced lines were designed represented by these pedigrees:
MTRaska*6///MT112219*4//Kronos994/Kronos562 and
MTRaska*6///MT112219*4//Kronos994/Kronos562/////MTRaska*5////
MTRaska//MT112219*4/ Kronos994/Kronos562///MT112219*5/Kronos(E529K)
An F_2 derived population of plants from the first pedigree were screened, and lines fixed for *tb-B1*-W341* were selected for increase. An F_4 derived population of plants from the second pedigree was screened, and lines fixed for *Rht-B1b*-E529K, *tb-A1*-W339* as well as the advantageous low cadmium allele *Low-Cd* were selected for increase. MT112219 contains the *Low-Cd* allele of the gene *Cdu-1*, while MT Raska does not (Zimmerl et al., 2014). Fixed genotype lines from each of these two populations were grown in single row plots. Three, top

yielding, genotypically fixed lines from each of the two single row advancements were selected for seed increase. Enhanced MT Raska background lines will be tested against elite durum varieties in state durum trials in 2025.

Conclusion

This research advances wheat breeding by investigating natural and induced mutant alleles that improve grain number, plant height, and tillering potential. Findings demonstrate that various alleles of *GNI-1, Rht-1,* and *TB1* can be used to optimize these traits, leading to enhanced grain yields. Findings provide direction for future research on these genes in wheat and other crops. Breeding populations and new lines are currently being developed to integrate demonstrated beneficial alleles into Montana breeding programs. Altogether, this research has and will continue to contribute to the development of germplasm that benefits Montana farmers and global wheat breeding efforts.

References

Hu, L.-M., Amoo, O., Zhai, Y.-G., Fan, C.-C., & Zhou, Y.-M. (2022). Regulation of shoot branching by BRANCHED1 in *Brassica napus* L. through gene editing technology [Original title in Chinese: 利用基因编辑技术研究BRANCHED1参与油菜分枝过程的调控]. *Biotechnology Bulletin*, (02), 1-6. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2021-1344

Shim, S., Ha, J., Kim, M. Y., Choi, M. S., Kang, S.-T., Jeong, S.-C., Moon, J.-K., & Lee, S.-H. (2019). *GmBRC1* is a Candidate Gene for Branching in Soybean (*Glycine max* (L.) Merrill). *International Journal of Molecular Sciences*, *20*(1), 135. https://doi.org/10.3390/ijms20010135

Zimmerl, S., Lafferty, J., & Buerstmayr, H. (2014). Assessing diversity in T riticum durum cultivars and breeding lines for high versus low cadmium content in seeds using the CAPS marker usw47. *Plant Breeding*, *133*(6), 712-717. https://doi.org/10.1111/pbr.12218

Zhang, K., Zhang, J., Cui, C., Chai, L., Zheng, B., Jiang, L., & Li, H. (2025). The WRKY28-BRC1 Transcription Factor Module Controls Shoot Branching in Brassica napus. Plants, 14(3),486. https://doi.org/10.3390/plants14030486

Zhang, K., Zhang, J., Cui, C., Chai, L., Zheng, B., Jiang, L., & Li, H. (2025). The WRKY28-BRC1 Transcription Factor Module Controls Shoot Branching in Brassica napus. *Plants*, *14*(3),486. https://doi.org/10.3390/plants14030486

CUMULATIVE REFERENCES CITED

Agriculture and Agri-Food Canada (AAFC) (2024). *Canada: Outlook for Principal Field Crops*. A. a. A.-F. Canada. https://agriculture.canada.ca/en/sector/crops/reports-statistics/canada-outlook-principal-field-crops-2024-12-19

Aguilar-Martinez, J. A., Poza-Carrion, C., & Cubas, P. (2007). Arabidopsis BRANCHED1 acts as an integrator of branching signals within axillary buds. *Plant Cell*, *19*(2), 458-472. https://doi.org/10.1105/tpc.106.048934

Alderson, T. (1965). Chemically induced delayed germinal mutation in Drosophila. *Nature*, *207*(4993), 164-167. https://doi.org/10.1038/207164a0

Ali, M. A., Hussain, M., Khan, M. I., Ali, Z., Zulkiffal, M., Anwar, J., . . . Zeeshan, M. (2010). Source-Sink Relationship between Photosynthetic Organs and Grain Yield Attributes during Grain Filling Stage in Spring Wheat (Triticum aestivum). *International Journal of Agriculture and Biology*, *12*(4), 509-515.

Alqudah, A. M., Koppolu, R., Wolde, G. M., Graner, A., & Schnurbusch, T. (2016). The Genetic Architecture of Barley Plant Stature. *Frontiers in Genetics*, *7*. https://doi.org/10.3389/fgene.2016.00117

Anderson, W., & Impiglia, A. (2002). Management of dryland wheat. *Wheat: Improvement and Production. Plant Production and Protection Series*, *30*, 567.

Appleford, N. E. J., Wilkinson, M. D., Ma, Q., Evans, D. J., Stone, M. C., Pearce, S. P.,…Lenton, J. R. (2007). Decreased shoot stature and grain α-amylase activity following ectopic expression of a gibberellin 2-oxidase gene in transgenic wheat. *Journal of Experimental Botany*, *58*(12), 3213-3226. https://doi.org/10.1093/jxb/erm166

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

Baasandorj, T., Ohm, J. B., Manthey, F., & Simsek, S. (2015). Effect of Kernel Size and Mill Type on Protein, Milling Yield, and Baking Quality of Hard Red Spring Wheat. *Cereal Chemistry*, *92*(1), 81-87. https://doi.org/10.1094/Cchem-12-13-0259-R

Bazargani, M. M., Sarhadi, E., Bushehri, A.-A. S., Matros, A., Mock, H.-P., Naghavi, M.-R.,…Moradi, F. (2011). A proteomics view on the role of drought-induced senescence and oxidative stress defense in enhanced stem reserves remobilization in wheat. *Journal of proteomics*, *74*(10), 1959-1973. https://doi.org/10.1016/j.jprot.2011.05.015

Beres, B. L., Rahmani, E., Clarke, J. M., Grassini, P., Pozniak, C. J., Geddes, C. M.,…Ransom, J. K. (2020). A Systematic Review of Durum Wheat: Enhancing Production Systems by Exploring Genotype, Environment, and Management (G x E x M) Synergies. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.568657

Blake, N. K., Stougaard, R. N., Bohannon, B., Weaver, D. K., Heo, H. Y., Lamb, P. F., . . . Talbert, L. E. (2014). Registration of 'Egan' Wheat with Resistance to Orange Wheat Blossom Midge. *Journal of Plant Registrations*, *8*(3), 298-302. https://doi.org/10.3198/jpr2014.04.0022crc

Boden, S. A., Cavanagh, C., Cullis, B. R., Ramm, K., Greenwood, J., Finnegan, E. J.,…Swain, S. M. (2015). Ppd-1 is a key regulator of inflorescence architecture and paired spikelet development in wheat. *Nature Plants*, *1*(2), 1-6. https://doi.org/10.1038/Nplants.2014.16

Bolle, C. (2004). The role of GRAS proteins in plant signal transduction and development. *Planta*, *218*, 683-692. https://doi.org/10.1007/s00425-004-1203-z

Bolser, D. M., Staines, D. M., Perry, E., & Kersey, P. J. (2017). Ensembl Plants: Integrating Tools for Visualizing, Mining, and Analyzing Plant Genomic Data. In (pp. 1-31). Springer New York. https://doi.org/10.1007/978-1-4939-6658-5_1

Borrill, P., Ramirez-Gonzalez, R., & Uauy, C. (2016). expVIP: a Customizable RNA-seq Data Analysis and Visualization Platform *Plant Physiology*, *170*(4), 2172-2186. https://doi.org/10.1104/pp.15.01667

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

Bruckner, P. L., Berg, J. E., Lamb, P. F., Carr, P., Wichman, D. M., Miller, J. H.,…Chen, X. (2019). Registration of 'Ray' Hard Red Winter Wheat. *Journal of Plant Registrations*, *13*(3), 392-395. https://doi.org/10.3198/jpr2019.03.0012crc

Bruckner, P. L., Berg, J. E., Riveland, N., Eckhoff, J. L., Wichman, D. M., Kephart, K. D., . . . Chen, X. (2007). Registration of 'Yellowstone' Wheat. *Journal of Plant Registrations*, *1*(1), 18-19. https://doi.org/10.3198/jpr2006.12.0788crc

Bruckner, P. L., & Frohberg, R. C. (1987). Rate and Duration of Grain Fill in Spring Wheat. *Crop Science*, *27*(3), 451-455. https://doi.org/DOI 10.2135/cropsci1987.0011183X002700030005x

Bull, H., Casao, M. C., Zwirek, M., Flavell, A. J., Thomas, W. T. B., Guo, W. B., . . . Waugh, R. (2017). Barley SIX-ROWED SPIKE3 encodes a putative Jumonji C-type H3K9me2/me3 demethylase that represses lateral spikelet fertility. *Nature Communications*, *8*. https://doi.org/10.1038/s41467-017-00940-7

Calderini, D. F., Castillo, F. M., Arenas-M, A., Molero, G., Reynolds, M. P., Craze, M., . . . McQueen-Mason, S. J. (2021). Overcoming the trade-off between grain weight and number in wheat by the ectopic expression of expansin in developing seeds leads to increased yield potential. *New Phytologist*, *230*(2), 629-640. https://doi.org/10.1111/nph.17048

Cao, S., Xu, D., Hanif, M., Xia, X., & He, Z. (2020). Genetic architecture underpinning yield component traits in wheat. *Theoretical and Applied Genetics*, *133*(6), 1811-1823. https://doi.org/10.1007/s00122-020-03562-8

Carver, B., Khalil, I., Krenzer, E., & MacKown, C. (2001). Breeding winter wheat for a dual-purpose management system. *Euphytica*, *119*, 231-234. https://doi.org/10.1023/A:1017543800311

Casebow, R., Hadley, C., Uppal, R., Addisu, M., Loddo, S., Kowalski, A.,…Gooding, M. (2016). Reduced Height (*Rht*) Alleles Affect Wheat Grain Quality. *Plos One*, *11*(5). https://doi.org/10.1371/journal.pone.0156056

Cecava, M. J. (1995). Making hay and haylage. In *Beef cattle feeding and nutrition* (pp. 104-116). Elsevier. https://doi.org/10.1016/B978-012552052-2/50011-1

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

Chandler, P. M., Harding, C. A., Ashton, A. R., Mulcair, M. D., Dixon, N. E., & Mander, L. N. (2008). Characterization of gibberellin receptor mutants of barley (*Hordeum vulgare* L.). *Molecular Plant*, *1*(2), 285-294. https://doi.org/10.1093/mp/ssn002

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

Christiansen, S., Svejcar, T., & Phillips, W. (1989). Spring and fall cattle grazing effects on components and total grain yield of winter wheat. *Agronomy Journal*, *81*(2), 145-150. https://doi.org/10.2134/agronj1989.00021962008100020002x

Clements, R., Cross, R., & Sanders, P. (1974). Effect of sowing rate on the growth and yield of standard and semidwarf wheat cultivars. *New Zealand journal of experimental agriculture*, *2*(2), 139-144. https://doi.org/10.1080/03015521.1974.10425750

Colbert, T., Till, B. J., Tompa, R., Reynolds, S., Steine, M. N., Yeung, A. T.,…Henikoff, S. (2001). High-throughput screening for induced point mutations. *Plant physiology*, *126*(2), 480-484. https://doi.org/10.1104/pp.126.2.480

Connaway, J. W., & Uren, A. (1935). *Actinomycosis in cattle: lump jaw, big jaw, and wooden tongue*. University of Missouri, College of Agriculture, Agricultural Experiment Station.

Cook, J., Acharya, R., Martin, J., Blake, N., Khan, I., Heo, H. Y.,…Sherman, J. (2021). Genetic analysis of stay-green, yield, and agronomic traits in spring wheat. *Crop Science*, *61*(1), 383-395. https://doi.org/10.1002/csc2.20302

Cook, J. P., Heo, H. Y., Varella, A. C., Lanning, S. P., Blake, N. K., Sherman, J. D., . . . Talbert, L. E. (2018). Evaluation of a QTL Mapping Population Composed of Hard Red Spring and Winter Wheat Alleles Using Various Marker Platforms. *Crop Science*, *58*(2), 701-712. https://doi.org/10.2135/cropsci2017.08.0488

Da Graça, J., Louzada, E., & Sauls, J. (2004). The origins of red pigmented grapefruits and the development of new varieties. Proceedings of the International Society of Citriculture,

De Vita, P., & Taranto, F. (2019). Durum wheat (Triticum turgidum ssp. durum) breeding to meet the challenge of climate change. In *Advances in Plant Breeding Strategies: Cereals: Volume 5* (pp. 471-524). Springer. https://doi.org/10.1007/978-3-030-23108-8_13

Dill, A., Thomas, S. G., Hu, J. H., Steber, C. M., & Sun, T. P. (2004). The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell*, *16*(6), 1392-1405. https://doi.org/10.1105/tpc.020958

Dixon, L. E., Greenwood, J. R., Bencivenga, S., Zhang, P., Cockram, J., Mellers, G.,…Boden, S. A. (2018). TEOSINTE BRANCHED1 Regulates Inflorescence Architecture and Development in Bread Wheat (Triticum aestivum). *Plant Cell*, *30*(3), 563-581. https://doi.org/10.1105/tpc.17.00961

Dixon, L. E., Pasquariello, M., & Boden, S. A. (2020). TEOSINTE BRANCHED1 regulates height and stem internode length in bread wheat. *Journal of Experimental Botany*, *71*(16), 4742-4750. https://doi.org/10.1093/jxb/eraa252

Dobrovolskaya, O., Martinek, P., Voylokov, A. V., Korzun, V., Roder, M. S., & Borner, A. (2009). Microsatellite mapping of genes that determine supernumerary spikelets in wheat (T. aestivum) and rye (S. cereale). *Theoretical and Applied Genetics*, *119*(5), 867-874. https://doi.org/10.1007/s00122-009-1095-1

Dobrovolskaya, O., Pont, C., Sibout, R., Martinek, P., Badaeva, E., Murat, F.,…Gautier, N. (2015). FRIZZY PANICLE drives supernumerary spikelets in bread wheat. *Plant Physiology*, *167*(1), 189-199. https://doi.org/10.1104/pp.114.250043

Dobrovolskaya, O. B. (2020). Supernumerary Spikelet Wheat Forms as Models for Studying Genetic Regulation of Inflorescence Development. *Russian Journal of Genetics*, *56*(11), 1298-1307. https://doi.org/10.1134/s1022795420110034

Doebley, J., Stec, A., & Gustus, C. (1995). Teosinte Branched1 and the Origin of Maize - Evidence for Epistasis and the Evolution of Dominance. *Genetics*, *141*(1), 333-346. https://doi.org/10.1093/genetics/141.1.333

Doebley, J., Stec, A., & Hubbard, L. (1997). The evolution of apical dominance in maize. *Nature*, *386*(6624), 485-488. https://doi.org/10.1038/386485a0

Donald, C. (1981). 14 COMPETITIVE PLANTS, COMMUNAL PLANTS, AND YIELD IN WHEAT CROPS. *Wheat science-today and tomorrow*, 223.

Donald, C. t. (1968). The breeding of crop ideotypes. *Euphytica*, *17*, 385-403. https://doi.org/10.1007/BF00056241

Dong, Z. B., Xiao, Y. G., Govindarajulu, R., Feil, R., Siddoway, M. L., Nielsen, T., . . . Chuck, G. (2019). The regulatory landscape of a core maize domestication module controlling bud dormancy and growth repression. *Nature Communications*, *10*. https://doi.org/10.1038/s41467-019-11774-w

Dong, B., Zheng, X., Liu, H., Able, J. A., Yang, H., Zhao, H.,…Liu, M. (2017). Effects of drought stress on pollen sterility, grain yield, abscisic acid and protective enzymes in two

winter wheat cultivars. *Frontiers in Plant Science*, *8*, 1008. https://doi.org/10.3389/fpls.2017.01008

Dou, J., Yang, H., Sun, D., Yang, S., Sun, S., Zhao, S., . . . Yang, L. (2022). The branchless gene Clbl in watermelon encoding a TERMINAL FLOWER 1 protein regulates the number of lateral branches [Article]. *Theoretical and Applied Genetics*, *135*(1), 65-79. https://doi.org/10.1007/s00122-021-03952-6

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*(6213), 1258096. https://doi.org/10.1126/science.1258096

Duggan, B., Richards, R., Van Herwaarden, A., & Fettell, N. (2005). Agronomic evaluation of a tiller inhibition gene (tin) in wheat. I. Effect on yield, yield components, and grain protein. *Australian Journal of Agricultural Research*, *56*(2), 169-178.

Edwards, J., Carver, B., Horn, G., & Payton, M. (2011). Impact of dual-purpose management on wheat grain yield. *Crop Science*, *51*(5), 2181-2185. https://doi.org/10.2135/cropsci2011.01.0043

El Baidouri, M., Murat, F., Veyssiere, M., Molinier, M., Flores, R., Burlot, L.,…Salse, J. (2017). Reconciling the evolutionary origin of bread wheat (Triticum aestivum). *New Phytologist*, *213*(3), 1477-1486. https://doi.org/10.1111/nph.14113

El Hassouni, K., Alahmad, S., Belkadi, B., Filali-Maltouf, A., Hickey, L. T., & Bassi, F. M. (2018). Root System Architecture and Its Association with Yield under Different Water Regimes in Durum Wheat. *Crop Science*, *58*(6), 2331-2346. https://doi.org/10.2135/cropsci2018.01.0076

Elias, E. M., & Manthey, F. A. (2007). Registration of 'Divide' Durum Wheat. *Journal of Plant Registrations*, *1*(1), 7-8. https://doi.org/10.3198/jpr2006.05.0281crc

Elias, E. M., Manthey, F. A., & AbuHammad, W. A. (2015). Registration of 'Carpio' Durum Wheat. *Journal of Plant Registrations*, *9*(1), 78-82. https://doi.org/10.3198/jpr2014.05.0030crc

Ellis, M. H., Spielmeyer, W., Gale, K. R., Rebetzke, G. J., & Richards, R. A. (2002). "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theoretical and Applied Genetics*, *105*(6-7), 1038-1042. https://doi.org/10.1007/s00122-002-1048-4

Enghiad, A., Ufer, D., Countryman, A. M., & Thilmany, D. D. (2017). An Overview of Global Wheat Market Fundamentals in an Era of Climate Concerns. *International Journal of Agronomy*, *2017*. https://doi.org/10.1155/2017/3931897

Erenstein, O., Poole, N., & Donovan, J. (2022). Role of staple cereals in human nutrition: Separating the wheat from the chaff in the infodemics age. *Trends in Food Science & Technology*, *119*, 508-513. https://doi.org/10.1016/j.tifs.2021.11.033

Evans, L. T. (1998). *Feeding the Ten Billion: Plants and Population Growth*. Cambridge University Press.

Feiz, L., Martin, J., & Giroux, M. (2009). Creation and functional analysis of new Puroindoline alleles in Triticum aestivum. *Theoretical and Applied Genetics*, *118*(2), 247-257. https://doi.org/10.1007/s00122-008-0893-1

Ferrante, A., Savin, R., & Slafer, G. A. (2020). Floret development and spike fertility in wheat: Differences between cultivars of contrasting yield potential and their sensitivity to photoperiod and soil N. *Field Crops Research*, *256*. https://doi.org/10.1016/j.fcr.2020.107908

Ficco, D. B. M., Beleggia, R., Pecorella, I., Giovanniello, V., Frenda, A. S., & Vita, P. D. (2020). Relationship between Seed Morphological Traits and Ash and Mineral Distribution along the Kernel Using Debranning in Durum Wheats from Different Geographic Sites. *Foods*, *9*(11), 1523. https://doi.org/10.3390/foods9111523

Fick, G. N., & Qualset, C. O. (1976). Seedling Emergence, Coleoptile Length, and Plant Height Relationships in Crosses of Dwarf and Standard-Height Wheats. *Euphytica*, *25*(3), 679-684. https://doi.org/10.1007/BF00041606

Flintham, J. E., Borner, 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

Fohner, G. (2002, December). Harvesting maximum value from small grain cereal forages. In *Proceedings of the 32nd California alfalfa and forage symposium, Reno, Nevada'.(Department of Agronomy and Range Science Extension, University of California: Davis) Available at http://alfalfa.ucdavis.edu/*

Foster, C. A. (1977). Slender: An accelerated extension growth mutant of barley. Barley Genetics Newsletter, 7(24–27).

Fouli, Y., Duiker, S., Fritton, D., Hall, M., Watson, J., & Johnson, D. (2012). Double cropping effects on forage yield and the field water balance. *Agricultural Water Management*, *115*, 104-117. https://doi.org/10.1016/j.agwat.2012.08.014

Fujioka, S., Yamane, H., Spray, C. R., Katsumi, M., Phinney, B. O., Gaskin, P.,…Takahashi, N. (1988). The dominant non-gibberellin-responding dwarf mutant (D8) of maize accumulates native gibberellins. *Proceedings of the National Academy of Sciences*, *85*(23), 9031-9035. https://doi.org/10.1073/pnas.85.23.9031

Gale, M. D., Law, C. N., & Worland, A. J. (1975). Chromosomal Location of a Major Dwarfing Gene from Norin 10 in New British Semi-Dwarf Wheats. *Heredity*, *35*(Dec), 417-421. https://doi.org/10.1038/hdy.1975.112

Gale, M. D., Marshall, G. A., & Rao, M. V. (1981). A Classification of the Norin 10 and Tom Thumb Dwarfing Genes in British, Mexican, Indian and Other Hexaploid Bread Wheat-Varieties. *Euphytica*, *30*(2), 355-361. https://doi.org/10.1007/BF00033997

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

Gasperini, D., Greenland, A., Hedden, P., Dreos, R., Harwood, W., & Griffiths, S. (2012). Genetic and physiological analysis of Rht8 in bread wheat: an alternative source of semi-dwarfism with a reduced sensitivity to brassinosteroids. *Journal of experimental botany*, *63*(12), 4419. https://doi.org/10.1093/jxb/ers138

Gaudin, A. C. M., McClymont, S. A., Soliman, S. S. M., & Raizada, M. N. (2014). The effect of altered dosage of a mutant allele of Teosinte branched 1 (tb1-ref) on the root system of modern maize. *Bmc Genetics*, *15*. https://doi.org/10.1186/1471-2156-15-23

Gauley, A., & Boden, S. A. (2019). Genetic pathways controlling inflorescence architecture and development in wheat and barley. *Journal of Integrative Plant Biology*, *61*(3), 296-309. https://doi.org/10.1111/jipb.12732

Greene, E. A., Codomo, C. A., Taylor, N. E., Henikoff, J. G., Till, B. J., Reynolds, S. H.,…Odden, A. R. (2003). Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. *Genetics*, *164*(2), 731-740. https://doi.org/10.1093/genetics/164.2.731

Golan, G., Ayalon, I., Perry, A., Zimran, G., Ade-Ajayi, T., Mosquna, A.,…Peleg, Z. (2019). GNI-A1 mediates trade-off between grain number and grain weight in tetraploid wheat. *Theoretical and Applied Genetics*, *132*(8), 2353-2365. https://doi.org/10.1007/s00122-019-03358-5

Gonzalez-Grandio, E., Pajoro, A., Franco-Zorrilla, J. M., Tarancon, C., Immink, R. G. H., & Cubas, P. (2017). Abscisic acid signaling is controlled by a BRANCHED1/HD-ZIP I cascade in Arabidopsis axillary buds. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(2), E245-E254. https://doi.org/10.1073/pnas.1613199114

Gordon, C., Derbyshire, J., Wiseman, H., Kane, E., & Melin, C. (1961). Preservation and feeding value of alfalfa stored as hay, haylage, and direct-cut silage. *Journal of Dairy Science*, *44*(7), 1299-1311. https://doi.org/10.3168/jds.S0022-0302(61)89881-0

Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z. L., Powers, S. J.,…Thomas, S. G. (2007). Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis (vol 18, pg 3399, 2006). *Plant Cell*, *19*(2), 726-726. https://doi.org/10.1105/tpc.107.190261

Guo, Z., Chen, D., Alqudah, A. M., Röder, M. S., Ganal, M. W., & Schnurbusch, T. (2017). Genome-wide association analyses of 54 traits identified multiple loci for the determination of floret fertility in wheat. *New Phytologist*, *214*(1), 257-270. https://doi.org/10.1111/nph.14342

Gutierrez-Gonzalez, J. J., Mascher, M., Poland, J., & Muehlbauer, G. J. (2019). Dense genotyping-by-sequencing linkage maps of two Synthetic W7984×Opata reference populations provide insights into wheat structural diversity. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-018-38111-3

Hale, C., Tillett, B., Martin, J., Hogg, A., Cook, J., & Giroux, M. (2024). The Grain Number Increase 1 alleles GNI-A1-105Y and-105K increase grain number in spring wheat. *Crop Science*. https://doi.org/10.1002/csc2.21267

Harberd, N. P., & Freeling, M. (1989). Genetics of dominant gibberellin-insensitive dwarfism in maize. *Genetics*, *121*(4), 827-838. https://doi.org/10.1093/genetics/121.4.827

Harrison, M. T., Evans, J. R., Dove, H., & Moore, A. D. (2011). Dual-purpose cereals: can the relative influences of management and environment on crop recovery and grain yield be dissected? *Crop and Pasture Science*, *62*(11), 930-946. https://doi.org/10.1071/CP11066

Hauvermale, A. L., Ariizumi, T., & Steber, C. M. (2012). Gibberellin Signaling: A Theme and Variations on DELLA Repression. *Plant Physiology*, *160*(1), 83-92. https://doi.org/10.1104/pp.112.200956

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

Hensz, R. A. (1985). 'Rio Red,' a new grapefruit with a deep-red colour. *Journal of the Rio Grande Valley Horticultural Society,* 38, 75–76.

Heo, H.-Y., Blake, N., Lanning, S., Lamb, P., Nash, D., Wichman, D.,…Reddy, G. (2018). Registration of 'NS Presser CLP'Hard Red Spring Wheat. *Journal of Plant Registrations*, *12*(1), 70-73. https://doi.org/10.3198/jpr2017.02.0008crc

Heo, H. Y., Lanning, S. P., Lamb, P. F., Nash, D., Wichman, D. M., Kephart, K. D., . . . Talbert, L. E. (2016). Registration of 'Lanning' Hard Red Spring Wheat. *Journal of Plant Registrations*, *10*(3), 287-290. https://doi.org/10.3198/jpr2016.03.0016crc

Hogg, A. C., Carr, P., Eberly, J., Chen, C. C., Kowatch-Carlson, C., Crutcher, F.,…Giroux, M. (2022). Registration of 'Lustre' durum wheat. *Journal of Plant Registrations*, *16*(3), 576-584. https://doi.org/10.1002/plr2.20214

Hogg, A. C., Hale, C. O., Tillett, B. J., Huang, L., Carr, P. M., Eberly, J.,…Giroux, M.J. (2025). Registration of 'MT Blackbeard'and 'MT Raska'durum wheat. *Journal of Plant Registrations*, *19*(1), e20425. https://doi.org/10.1002/plr2.20425

Hogg, A. C., & Giroux, M. J. (2019). Milling and baking quality of hexaploid spring wheat starch synthase IIa (ssIIa) mutants with elevated amylose content. *Cereal Chemistry*, *96*(3), 532-544. https://doi.org/10.1002/cche.10153

Holman, J. D., Thompson, C. R., Hale, R. L., & Schlegel, A. J. (2009). Grazing effects on yield and quality of hard red and hard white winter wheat. *Agronomy Journal*, *101*(4), 775-788. https://doi.org/10.2134/agronj2008.0163x

Hoogendoorn, J., Rickson, J. M., & Gale, M. D. (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

Hu, L.-M., Amoo, O., Zhai, Y.-G., Fan, C.-C., & Zhou, Y.-M. (2022). Regulation of shoot branching by BRANCHED1 in *Brassica napus* L. through gene editing technology [Original title in Chinese: 利用基因编辑技术研究BRANCHED1参与油菜分枝过程的调控]. *Biotechnology Bulletin*, (02), 1-6. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2021-1344

Ikeda, A., Ueguchi-Tanaka, M., Sonoda, Y., Kitano, H., Koshioka, M., Futsuhara, Y.,…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

Ishag, H., & Taha, M. (1974). Production and survival of tillers of wheat and their contribution to yield. *The Journal of Agricultural Science*, *83*(1), 117-124. https://doi.org/10.1017/S0021859600047079

Ishizaki, T., Ueda, Y., Takai, T., Maruyama, K., & Tsujimoto, Y. (2023). In-frame mutation in rice *TEOSINTE BRANCHED1 (OsTB1)* improves productivity under phosphorus deficiency. *Plant Science*, *330*, 111627. https://doi.org/10.1016/j.plantsci.2023.111627

Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M., & Matsuoka, M. (2002). The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. *Plant Cell*, *14*(1), 57-70. https://doi.org/10.1105/tpc.010319

Jafari, F., Wang, B., Wang, H., & Zou, J. (2024). Breeding maize of ideal plant architecture for high-density planting tolerance through modulating shade avoidance response and beyond. *Journal of Integrative Plant Biology*, *66*(5), 849-864. https://doi.org/10.1111/jipb.13603

Jankowicz-Cieslak, J., & Till, B. J. (2016). Chemical mutagenesis of seed and vegetatively propagated plants using EMS. *Current protocols in plant biology*, *1*(4), 617-635. https://doi.org/10.1002/cppb.20040

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

Jobson, E. M., Martin, J. M., Sharrock, R., Hogg, A. C., & Giroux, M. J. (2021). Identification and molecular characterization of novel *Rht-1* alleles in hard red spring wheat. *Crop Science*, *61*(2), 1030-1037. https://doi.org/10.1002/csc2.20375

Jones, B. H., Blake, N. K., Heo, H. Y., Martin, J. M., Torrion, J. A., & Talbert, L. E. (2021). Allelic response of yield component traits to resource availability in spring wheat. *Theoretical and Applied Genetics*, *134*(2), 603-620. https://doi.org/10.1007/s00122-020-03717-7

Joppa, L. R., Du, C. H., Hart, G. E., & Hareland, G. A. (1997). Mapping gene(s) for grain protein in tetraploid wheat (Triticum turgidum L.) using a population of recombinant inbred chromosome lines. *Crop Science*, *37*(5), 1586-1589. https://doi.org/10.2135/cropsci1997.0011183X003700050030x

Khaliq, I., Irshad, A., & Ahsan, M. (2008). Awns and flag leaf contribution towards grain yield in spring wheat (Triticum aestivum L.). *Cereal Research Communications*, *36*(1), 65-76. https://doi.org/10.1556/Crc.36.2008.1.7

Khush, G. S. (2001). Green revolution: the way forward. *Nature reviews genetics*, *2*(10), 815-822. https://doi.org/10.1038/35093585

Klein, H., Gallagher, J., Demesa-Arevalo, E., Abraham-Juarez, M. J., Heeney, M., Feil, R., . . . Bartlett, M. (2022). Recruitment of an ancient branching program to suppress carpel development in maize flowers. *Proc Natl Acad Sci U S A*, *119*(2). https://doi.org/10.1073/pnas.2115871119

Komatsuda, T., Pourkheirandish, M., He, C., Azhaguvel, P., Kanamori, H., Perovic, D.,…Tagiri, A. (2007). Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene. *Proceedings of the National Academy of Sciences*, *104*(4), 1424-1429. https://doi.org/10.1073/pnas.0608580104

Krasileva, K. V., Vasquez-Gross, H. A., Howell, T., Bailey, P., Paraiso, F., Clissold, L.,…Dubcovsky, J. (2017). Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(6), E913-E921. https://doi.org/10.1073/pnas.1619268114

Koorneef, M., Elgersma, A., Hanhart, C. v., van Loenen-Martinet, E., Van Rijn, L., & Zeevaart, J. (1985). A gibberellin insensitive mutant of Arabidopsis thaliana. *Physiologia Plantarum*, *65*(1), 33-39. https://doi.org/10.1111/j.1399-3054.1985.tb02355.x

Kukal, M. S., & Irmak, S. (2018). US agro-climate in 20th century: Growing degree days, first and last frost, growing season length, and impacts on crop yields. *Scientific reports*, *8*(1), 6977. https://doi.org/10.1038/s41598-018-25212-2

Lanning, S., Carlson, G., Lamb, P., Nash, D., Wichman, D., Kephart, K.,…Eckhoff, J. (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.,…Talbert, L. E. (2006). Registration of 'Vida' wheat. *Crop Science*, *46*(5), 2315-2316. https://doi.org/10.2135/cropsci2006.03.0167

Lanning, S., Martin, J., Stougaard, R., Guillen-Portal, F., Blake, N., Sherman, J.,…Carlson, G. (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

Lanning, S., Carlson, G., Nash, D., Wichman, D., Kephart, K., Stougaard, R.,…Talbert, L. (2004). Registration of 'Choteau' wheat. *Crop science*, *44*(6), 2264-2266.

Lanning, S., Talbert, L., McGuire, C., Bowman, H., Carlson, G., Jackson, G.,…Stallknecht, G. (1994). Registration of 'McNeal' wheat. https://doi.org/10.2135/cropsci1994.0011183X003400040060x

Lewis, J. M., Mackintosh, C. A., Shin, S., Gilding, E., Kravchenko, S., Baldridge, G.,…Muehlbauer, G. J. (2008). Overexpression of the maize Teosinte Branched1 gene in wheat suppresses tiller development. *Plant Cell Reports*, *27*(7), 1217-1225. https://doi.org/10.1007/s00299-008-0543-8

Liatukas, Z., & Ruzgas, V. (2011). Coleoptile length and plant height of modern tall and semi-dwarf European winter wheat varieties. *Acta Societatis Botanicorum Poloniae*, *80*(3), 197-203. https://doi.org/10.5586/asbp.2011.018

Li, A., Yang, W., Lou, X., Liu, D., Sun, J., Guo, X.,…Ling, H. Q. (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

Li, L. L., Bian, Y. J., Dong, Y., Song, J., Liu, D., Zeng, J. Q., . . . Cao, S. H. (2023). Identification and validation of stable quantitative trait loci for yield component traits in wheat. *Crop Journal*, *11*(2), 558-563. https://doi.org/10.1016/j.cj.2022.09.012

Li, X.-P., Lan, S.-Q., Liu, Y.-P., Gale, M., & Worland, T. (2006). Effects of different Rht-B1b, Rht-D1b and Rht-B1c dwarfing genes on agronomic characteristics in wheat. *Cereal research communications*, *34*(2-3), 919-924. https://doi.org/10.1556/crc.34.2006.2-3.220

Lin, Y., Jiang, X. J., Hu, H. Y., Zhou, K. Y., Wang, Q., Yu, S. F., . . . Liu, Y. X. (2021). QTL mapping for grain number per spikelet in wheat using a high-density genetic map. *Crop Journal*, *9*(5), 1108-1114. https://doi.org/10.1016/j.cj.2020.12.006

Liu, C., Wang, J., Huang, T., Wang, F., Yuan, F., Cheng, X.,…Liu, K. (2010). A missense mutation in the VHYNP motif of a DELLA protein causes a semi-dwarf mutant phenotype in Brassica napus. *Theoretical and Applied Genetics*, *121*, 249-258. https://doi.org/10.1007/s00122-010-1306-9

Liu, Y., Zhang, P., Li, M., Chang, L., Cheng, H., Chai, S., & Yang, D. (2020). Dynamic responses of accumulation and remobilization of water soluble carbohydrates in wheat stem to drought stress. *Plant Physiology and Biochemistry*, *155*, 262-270. https://doi.org/10.1016/j.plaphy.2020.07.024

Lou, X. Y., Li, X., Li, A. X., Pu, M. Y., Shoaib, M., Liu, D. C.,…Yang, W. L. (2016). Molecular Characterization of Three *GIBBERELLIN-INSENSITIVE DWARF2* Homologous Genes in Common Wheat. *Plos One*, *11*(6). https://doi.org/10.1371/journal.pone.0157642

Lizana, X. C., Riegel, R., Gomez, L. D., Herrera, J., Isla, A., McQueen-Mason, S. J., & Calderini, D. F. (2010). Expansins expression is associated with grain size dynamics in wheat (Triticum aestivum L.). *Journal of Experimental Botany*, *61*(4), 1147-1157. https://doi.org/10.1093/jxb/erp380

Marshall, D. R., Mares, D. J., Moss, H. J., & Ellison, F. W. (1986). Effects of Grain Shape and Size on Milling Yields in Wheat .2. Experimental Studies. *Australian Journal of Agricultural Research*, *37*(4), 331-342. https://doi.org/10.1071/AR9860331

McCallum, C. M., Comai, L., Greene, E. A., & Henikoff, S. (2000). Targeted screening for induced mutations. *Nature biotechnology*, *18*(4), 455-457. https://doi.org/10.1038/74542

McVittie, J. A., Gale, M. D., Marshall, G. A., & Westcott, B. (1978). The intra-chromosomal mapping of the Norin 10 and Tom Thumb dwarfing genes. *Heredity*, *40*(1), 67-70. https://doi.org/10.1038/hdy.1978.8

Maluszynski, M., Nichterlein, K., Van Zanten, L., & Ahloowalia, B. (2000). Officially released mutant varieties-the FAO/IAEA Database. *Plant Cell, Tissue and Organ Culture*, *65*(3), 175-177.

Martín-Trillo, M., & Cubas, P. (2010). TCP genes: a family snapshot ten years later. *Trends in plant science*, *15*(1), 31-39.

Mathews, K. L., Chapman, S. C., Trethowan, R., Singh, R. P., Crossa, J., Pfeiffer, W.,…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

Mizuno, N., Ishikawa, G., Kojima, H., Tougou, M., Kiribuchi-Otobe, C., Fujita, M., & Nakamura, K. (2021). Genetic mechanisms determining grain number distribution along the spike and their effect on yield components in wheat. *Molecular Breeding*, *41*(10). https://doi.org/10.1007/s11032-021-01255-8

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*. https://doi.org/10.1186/s12870-018-1465-4

Mock, J., & Pearce, R. (1975). An ideotype of maize. *Euphytica*, *24*(3), 613-623. https://doi.org/10.1007/BF00132898

Mueller, B., Hauser, M., Iles, C., Rimi, R. H., Zwiers, F. W., & Wan, H. (2015). Lengthening of the growing season in wheat and maize producing regions. *Weather and Climate Extremes*, *9*, 47-56. https://doi.org/10.1016/j.wace.2015.04.001

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

Naruoka, Y., Talbert, L. E., Lanning, S. P., Blake, N. K., Martin, J. M., & Sherman, J. D. (2011). Identification of quantitative trait loci for productive tiller number and its relationship to agronomic traits in spring wheat. *Theoretical and Applied Genetics*, *123*(6), 1043-1053. https://doi.org/10.1007/s00122-011-1646-0

Nasseer, A., Martin, J., Heo, H., Blake, N., Sherman, J., Pumphrey, M.,…Talbert, L. (2016). Impact of a quantitative trait locus for tiller number on plasticity of agronomic traits in spring wheat. *Crop Science*, *56*(2), 595-602. https://doi.org/10.2135/cropsci2015.05.0325

NCEI (National Centers for Environmental Information). (2025). Daily summaries station details: Bozeman 6 W Experimental Farm, MT US, GHCND:USC00241047. Climate Data Online (CDO) | National Centers for Environmental Information (NCEI). https://www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00241047/detail

NCEI (National Centers for Environmental Information). (2025). Daily summaries station details: Havre Airport ASOS, MT US, GHCND:USW00094012. Climate Data Online (CDO) | National Centers for Environmental Information (NCEI). https://www.ncei.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USW00094012/detail

NCEI (National Centers for Environmental Information). (2025). Daily summaries station details: Sidney, MT US, GHCND:USC00247560. Climate Data Online (CDO) | National Centers for Environmental Information (NCEI). https://www.ncei.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00247560/detail

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A.,…Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology & Biotechnological Equipment*, *30*(1), 1-16. https://doi.org/10.1080/13102818.2015.1087333

O'Keefe, C., Penner, G., McKinnon, J., Larson, K., Damiran, D., & Lardner, H. B. (2022). Effects of stage of maturity at harvest of cereal crops on biomass and quality, estimated forage dry matter intake, beef cow performance, and system economics. *Applied Animal Science*, *38*(6), 607-619. https://doi.org/10.15232/aas.2022-02327

Pandey, M., Singh, A. K., DePauw, R. M., Bokore, F. E., Ellouze, W., Knox, R. E., & Cuthbert, R. D. (2015). Coleoptile length, gibberellin sensitivity, and plant height variation of durum wheat in Canada. *Canadian Journal of Plant Science*, *95*(6), 1259-1264. https://doi.org/10.4141/cjps-2015-154

Pearce, S., Saville, R., Vaughan, S. P., Chandler, P. M., Wilhelm, E. P., Sparks, C. A.,…Phillips, A. L. (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.,…Pelica, F. (1999). 'Green revolution'genes encode mutant gibberellin response modulators. *nature*, *400*(6741), 256-261. https://doi.org/10.1038/22307

Peng, J. R., Carol, P., Richards, D. E., King, K. E., Cowling, R. J., Murphy, G. P., & Harberd, N. P. (1997). The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes & Development*, *11*(23), 3194-3205. https://doi.org/10.1101/gad.11.23.3194

Peng, M., Gao, M., Abdel-Aal, E. S. M., Hucl, P., & Chibbar, R. N. (1999). Separation and characterization of A- and B-type starch granules in wheat endosperm. *Cereal Chemistry*, *76*(3), 375-379. https://doi.org/10.1094/CCHEM.1999.76.3.375

Perotti, M. F., Ribone, P. A., & Chan, R. L. (2017). Plant transcription factors from the homeodomain-leucine zipper family I. Role in development and stress responses. *IUBMB Life*, *69*(5), 280-289. https://doi.org/10.1002/iub.1619

Peterson, J. (December 23, 2024). WMR-World Durum 2024. North Dakota Wheat Commission. https://www.ndwheat.com/post/wmr-world-durum-2024

Quintero, A., Molero, G., Reynolds, M. P., & Calderini, D. F. (2018). Trade-off between grain weight and grain number in wheat depends on GxE interaction: A case study of an elite CIMMYT panel (CIMCOG). *European Journal of Agronomy*, *92*, 17-29. https://doi.org/10.1016/j.eja.2017.09.007

Ramsay, L., Comadran, J., Druka, A., Marshall, D. F., Thomas, W. T. B., Macaulay, M.,…Waugh, R. (2011). INTERMEDIUM-C, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene TEOSINTE BRANCHED 1. *Nature Genetics*, *43*(2), 169-U125. https://doi.org/10.1038/ng.745

Rasheed, A., Wen, W., Gao, F., Zhai, S., Jin, H., Liu, J.,…Xia, X. (2016). Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. *Theoretical and Applied Genetics*, *129*, 1843-1860. https://doi.org/10.1007/s00122-016-2743-x

Rebetzke, G., Ellis, M., Bonnett, D., Condon, A., Falk, D., & Richards, R. (2011). The Rht13 dwarfing gene reduces peduncle length and plant height to increase grain number and yield of wheat. *Field Crops Research*, *124*(3), 323-331. https://doi.org/10.1016/j.fcr.2011.06.022

Rebetzke, G. J., Ellis, M. H., Bonnett, D. G., & Richards, R. A. (2007). Molecular mapping of genes for coleoptile growth in bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *114*(7), 1173-1183. https://doi.org/10.1007/s00122-007-0509-1

Redmon, L. A., Horn, G. W., Krenzer Jr, E. G., & Bernardo, D. J. (1995). A review of livestock grazing and wheat grain yield: Boom or bust? *Agronomy Journal*, *87*(2), 137-147. https://doi.org/10.2134/agronj1995.00021962008700020001x

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

Reynolds, M. P., Slafer, G. A., Foulkes, J. M., Griffiths, S., Murchie, E. H., Carmo-Silva, E., . . . Flavell, R. B. (2022). A wiring diagram to integrate physiological traits of wheat yield potential (vol 3, pg 318, 2022). *Nature Food*, *3*(8), 665-665. https://doi.org/10.1038/s43016-022-00574-z

Richards, R. (1988). A tiller inhibitor gene in wheat and its effect on plant growth. *Australian Journal of Agricultural Research*, *39*(5), 749-757. https://doi.org/10.1071/AR9880749

Richards, R. (1992). The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. *Australian Journal of Agricultural Research*, *43*(3), 517-527. https://doi.org/10.1071/AR9920517

Riede, C. R., & Anderson, J. A. (1996). Linkage of RFLP markers to an aluminum tolerance gene in wheat. *Crop Science*, *36*(4), 905-909. https://doi.org/10.2135/cropsci1996.0011183X0036000400015x

Rutger, J. (2009). The induced sd1 mutant and other useful mutant genes in modern rice varieties. *Induced plant mutations in the genomics era. Rome: Food and Agriculture Organization of the United Nations*, 44-47.

Rutger, J. N., Peterson, M. L., & Hu, C. (1977). Registration of Calrose 76 rice 1 (Reg. no. 45). *Crop Science*, *17*(6), 978-978. https://doi.org/10.2135/cropsci1977.0011183X001700060047x

Sakuma, S., Golan, G., Guo, Z. F., Ogawa, T., Tagiri, A., Sugimoto, K.,…Komatsuda, T. (2019). Unleashing floret fertility in wheat through the mutation of a homeobox gene. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(11), 5182-5187. https://doi.org/10.1073/pnas.1815465116

Sakuma, S., & Koppolu, R. (2023). Form follows function in Triticeae inflorescences. *Breeding Science*, *73*(1), 46-56. https://doi.org/10.1270/jsbbs.22085

Sakuma, S., Pourkheirandish, M., Hensel, G., Kumlehn, J., Stein, N., Tagiri, A., . . . Komatsuda, T. (2013). Divergence of expression pattern contributed to neofunctionalization of duplicated HD-Zip I transcription factor in barley. *New Phytologist*, *197*(3), 939-948. https://doi.org/10.1111/nph.12068

Sakuma, S., Pourkheirandish, M., Matsumoto, T., Koba, T., & Komatsuda, T. (2010). Duplication of a well-conserved homeodomain-leucine zipper transcription factor gene in barley generates a copy with more specific functions. *Functional & Integrative Genomics*, *10*(1), 123-133. https://doi.org/10.1007/s10142-009-0134-y

Sakuma, S., & Schnurbusch, T. (2020). Of floral fortune: tinkering with the grain yield potential of cereal crops. *New Phytologist*, *225*(5), 1873-1882. https://doi.org/10.1111/nph.16189

Scarascia Mugnozza, G. T. (2005). The contribution of Italian wheat geneticists: from Nazareno Strampelli to Francesco D'Amato. *Rome: Accademia Nazionale delle Scienze*, 53-75.

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. http://dx.doi.org/10.2134/agronj1998.00021962009000050002x

Schmidt, J. E., Bowles, T. M., & Gaudin, A. C. M. (2016). Using Ancient Traits to Convert Soil Health into Crop Yield: Impact of Selection on Maize Root and Rhizosphere Function. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00373

Sedgley, R. (1991). An appraisal of the Donald ideotype after 21 years. *Field Crops Research*, *26*(2), 93-112. https://doi.org/10.1016/0378-4290(91)90031-P

Semenov, M. A., & Stratonovitch, P. (2013). Designing high-yielding wheat ideotypes for a changing climate. *Food and Energy Security*, *2*(3), 185-196. https://doi.org/10.1002/fes3.34

Senapati, N., Stratonovitch, P., Paul, M. J., & Semenov, M. A. (2019). Drought tolerance during reproductive development is important for increasing wheat yield potential under climate change in Europe. *Journal of experimental botany*, *70*(9), 2549-2560. https://doi.org/10.1093/jxb/ery226

Serrago, R. A., Alzueta, I., Savin, R., & Slafer, G. A. (2013). Understanding grain yield responses to source-sink ratios during grain filling in wheat and barley under contrasting environments. *Field Crops Research*, *150*, 42-51.

Shang, Y., Yuan, L., Di, Z. C., Jia, Y., Zhang, Z. L., Li, S. J., . . . Li, C. D. (2020). A CYC/TB1-type TCP transcription factor controls spikelet meristem identity in barley. *Journal of Experimental Botany*, *71*(22), 7118-7131. https://doi.org/10.1093/jxb/eraa416

Shao, J., Haider, I., Xiong, L., Zhu, X., Hussain, R. M. F., Övernäs, E., . . . Ouwerkerk, P. B. F. (2018). Functional analysis of the HD-Zip transcription factor genes Oshox12 and Oshox14 in rice. *PLOS ONE*, *13*(7), e0199248. https://doi.org/10.1371/journal.pone.0199248

Sharma, R. (1993). Selection for biomass yield in wheat. *Euphytica*, *70*, 35-42. https://doi.org/10.1007/BF00029638

Sharrow, S., & Motazedian, I. (1987). Spring Grazing Effects on Components of Winter Wheat Yield 1. *Agronomy Journal*, *79*(3), 502-504. https://doi.org/10.2134/agronj1987.00021962007900030019x

Shim, S., Ha, J., Kim, M. Y., Choi, M. S., Kang, S.-T., Jeong, S.-C., Moon, J.-K., & Lee, S.-H. (2019). *GmBRC1* is a Candidate Gene for Branching in Soybean (*Glycine max* (L.) Merrill). *International Journal of Molecular Sciences*, *20*(1), 135. https://doi.org/10.3390/ijms20010135

Shimada, A., Ueguchi-Tanaka, M., Nakatsu, T., Nakajima, M., Naoe, Y., Ohmiya, H.,…Matsuoka, M. (2008). Structural basis for gibberellin recognition by its receptor GID1. *Nature*, *456*(7221), 520-U544. https://doi.org/10.1038/nature07546

Sigalas, P. P., Bennett, T., Buchner, P., Thomas, S. G., Jamois, F., Arkoun, M.,…Hawkesford, M. J. (2024). At the crossroads: strigolactones mediate changes in cytokinin synthesis and signalling in response to nitrogen limitation. *The Plant Journal*, *120*(1), 139-158. https://doi.org/10.1111/tpj.16976

Sikora, P., Chawade, A., Larsson, M., Olsson, J., & Olsson, O. (2011). Mutagenesis as a Tool in Plant Genetics, Functional Genomics, and Breeding. *International Journal of Plant Genomics*, *2011*, 1-13. https://doi.org/10.1155/2011/314829

Silverstone, A. L., & Sun, T. P. (2000). Gibberellins and the green revolution. *Trends in Plant Science*, *5*(1), 1-2. https://doi.org/Doi 10.1016/S1360-1385(99)01516-2 https://doi.org/10.1016/S1360-1385(99)01516-2

Sinclair, T. R., & No'am G, S. (1995). Global environment change and simulated forage quality of wheat I. Nonstressed conditions. *Field Crops Research*, *40*(1), 19-27. https://doi.org/10.1016/0378-4290(94)00091-P

Slafer, G. A., Foulkes, M. J., Reynolds, M. P., Murchie, E. H., Carmo-Silva, E., Flavell, R., . . . Griffiths, S. (2023). A 'wiring diagram' for sink strength traits impacting wheat yield potential. *Journal of Experimental Botany*, *74*(1), 40-71. https://doi.org/10.1093/jxb/erac410

Snowdon, R. J., Wittkop, B., Chen, T.-W., & Stahl, A. (2021). Crop adaptation to climate change as a consequence of long-term breeding. *Theoretical and Applied Genetics*, *134*(6), 1613-1623. https://doi.org/10.1007/s00122-020-03729-3

Sourdille, P., Charmet, G., Trottet, M., Tixier, M. H., Boeuf, C., Negre, S.,…Bernard, M. (1998). Linkage between RFLP molecular markers and the dwarfing genes Rht-B1 and Rht-D1 in wheat. *Hereditas*, *128*(1), 41-46. https://doi.org/10.1111/j.1601-5223.1998.00041.x

Studer, A., Zhao, Q., Ross-Ibarra, J., & Doebley, J. (2011). Identification of a functional transposon insertion in the maize domestication gene tb1. *Nature Genetics*, *43*(11), 1160-U1164. https://doi.org/10.1038/ng.942

Tabbita, F., Pearce, S., & Barneix, A. J. (2017). Breeding for increased grain protein and micronutrient content in wheat: Ten years of the GPC-B1 gene. *Journal of Cereal Science*, *73*, 183-191. https://doi.org/10.1016/j.jcs.2017.01.003

Takeda, T., Suwa, Y., Suzuki, M., Kitano, H., Ueguchi-Tanaka, M., Ashikari, M., . . . Ueguchi, C. (2003). The OsTB1 gene negatively regulates lateral branching in rice. *Plant Journal*, *33*(3), 513-520. https://doi.org/10.1046/j.1365-313X.2003.01648.x

Thirulogachandar, V., Alqudah, A. M., Koppolu, R., Rutten, T., Graner, A., Hensel, G., . . . Kuhlmann, M. (2017). Leaf primordium size specifies leaf width and vein number among

row-type classes in barley. *The Plant Journal*, *91*(4), 601-612. https://doi.org/10.1111/tpj.13590

Thomas, S. G. (2017). Novel *Rht-1* dwarfing genes: tools for wheat breeding and dissecting the function of DELLA proteins. *Journal of Experimental Botany*, *68*(3), 353-358. https://doi.org/10.1093/jxb/erw509

Thomashow, M. F. (1998). Role of cold-responsive genes in plant freezing tolerance. *Plant physiology*, *118*(1), 1-8. https://doi.org/10.1104/pp.118.1.1

Tillett, B. J., Hale, C. O., Martin, J. M., & Giroux, M. J. (2022). Genes Impacting Grain Weight and Number in Wheat (Triticum aestivum L. ssp. aestivum). *Plants-Basel*, *11*(13). https://doi.org/10.3390/plants11131772

Tokatlidis, I. (2014). Addressing the yield by density interaction is a prerequisite to bridge the yield gap of rain-fed wheat. *Annals of Applied Biology*, *165*(1), 27-42. https://doi.org/10.1111/aab.12121

Trethowan, R. M., Singh, R. P., Huerta-Espino, J., Crossa, J., & van Ginkel, M. (2001). Coleoptile length variation of near-isogenic *Rht* lines of modern CIMMYT bread and durum wheats. *Field Crops Research*, *70*(3), 167-176. https://doi.org/10.1016/S0378-4290(00)00153-2

Tsai, H., Howell, T., Nitcher, R., Missirian, V., Watson, B., Ngo, K. J.,…Comai, L. (2011). Discovery of Rare Mutations in Populations: TILLING by Sequencing. *Plant Physiology*, *156*(3), 1257-1268. https://doi.org/10.1104/pp.110.169748

Tuinstra, M. R., Ejeta, G., & Goldsbrough, P. B. (1997). Heterogeneous inbred family (HIF) analysis: a method for developing near-isogenic lines that differ at quantitative trait loci. *Theoretical and Applied Genetics*, *95*(5-6), 1005-1011. https://doi.org/10.1007/s001220050654

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M.,…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

U.S. Department of Agriculture (USDA). (2024). *Crops and crop products: Grains and oilseeds.* In *Small grains annual summary* (ID: 5t34sj573). United States Department of Agriculture, Economics, Statistics and Market Information System. https://usda.library.cornell.edu/concern/publications/5t34sj573

U.S. Department of Agriculture (USDA). (2024). Montana 2024 wheat varieties. U.S. Department of Agriculture, National Agricultural Statistics Service. https://www.nass.usda.gov/Statistics_by_State/Montana/index.php

U.S. Department of Agriculture (USDA). (2024). Production - Wheat. U.S. Department of Agriculture, Foreign Agricultural Service. Retrieved February 27, 2025, from https://www.fas.usda.gov/data/production/commodity/0410000

U.S. Department of Agriculture (USDA). (2024). Small grains 2024 summary. U.S. Department of Agriculture, Economics, Statistics, and Marketing Information System. https://usda.library.cornell.edu/concern/publications/5t34sj573

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

Volkman, M. M., Martin, J. M., Hogg, A. C., Wright, L., Hale, C., Carr, P. M., & Giroux, M. J. (2022). Durum wheat Teosinte Branched1 null mutations increase tillering. *Crop Science*, *62*(4), 1522-1530. https://doi.org/10.1002/csc2.20775

Walker, A. R., Lee, E., Bogs, J., McDavid, D. A., Thomas, M. R., & Robinson, S. P. (2007). White grapes arose through the mutation of two similar and adjacent regulatory genes. *The Plant Journal*, *49*(5), 772-785. https://doi.org/10.1111/j.1365-313X.2006.02997.x

Wang, H. W., Seiler, C., Sreenivasulu, N., Wiren, V., & Kuhlmann, M. (2022). INTERMEDIUM-C mediates the shade-induced bud growth arrest in barley. *Journal of Experimental Botany*, *73*(7), 1963-1977. https://doi.org/10.1093/jxb/erab542

Wang, M., Le Moigne, M.-A., Bertheloot, J., Crespel, L., Perez-Garcia, M.-D., Ogé, L.,…Sakr, S. (2019). BRANCHED1: A Key Hub of Shoot Branching. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.00076

Wang, Y., Du, F., Wang, J., Wang, K., Tian, C., Qi, X.,…Jiao, Y. (2022). Improving bread wheat yield through modulating an unselected AP2/ERF gene. *Nature Plants*, *8*(8), 930-939. https://doi.org/10.1038/s41477-022-01197-9

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S. C., Prasad, S. V. S., Rebetzke, G. J., . . . Watt, M. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany*, *63*(9), 3485-3498. https://doi.org/10.1093/jxb/ers111

Whan, B., Delane, R., & Gilmour, R. (1988). The potential of reduced tillering wheats in dry environments. Proceedings of the seventh international wheat genetics symposium, held at Cambridge, UK, 13-19 July 1988.

Williams, J. L., Sherman, J. D., Lamb, P., Cook, J., Lachowiec, J. A., & Bourgault, M. (2022). Relationships between roots, the stay-green phenotype, and agronomic performance in

barley and wheat grown in semi-arid conditions. *The Plant Phenome Journal*, *5*(1), e220050. https://doi.org/10.1002/ppj2.20050

Whipple, C. J., Kebrom, T. H., Weber, A. L., Yang, F., Hall, D., Meeley, R., . . . Jackson, D. P. (2011). grassy tillers1 promotes apical dominance in maize and responds to shade signals in the grasses. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(33), E506-E512.

Winter, S., & Musick, J. (1991). Grazed wheat grain yield relationships. *Agronomy Journal*, *83*(1), 130-135. https://doi.org/10.2134/agronj1991.00021962008300010030x

Xie, Q., Mayes, S., & Sparkes, D. L. (2015). Carpel size, grain filling, and morphology determine individual grain weight in wheat. *Journal of Experimental Botany*, *66*(21), 6715-6730. https://doi.org/10.1093/jxb/erv378

Xie, Q., & Sparkes, D. L. (2021). Dissecting the trade-off of grain number and size in wheat. *Planta*, *254*(1). https://doi.org/10.1007/s00425-021-03658-5

Xu, D. A., Bian, Y. J., Luo, X. M., Jia, C. F., Hao, Q. L., Tian, X. L.,…Cao, S. H. (2023). Dissecting pleiotropic functions of the wheat Green Revolution gene Rht1 in plant morphogenesis and yield formation. *Development*, *150*(20). https://doi.org/10.1242/dev.201601

Youssefian, S., Kirby, E. J. M., & Gale, M. D. (1992). Pleiotropic Effects of the Ga-Insensitive Rht Dwarfing Genes in Wheat .2. Effects on Leaf, Stem, Ear and Floret Growth. *Field Crops Research*, *28*(3), 191-210. https://doi.org/10.1016/0378-4290(92)90040-G

Yunusa, I., & Sedgley, R. (1992). Reduced Tillering Spring Wheats for Heavy Textured Soils in a Semi-arid Mediterranean Environment. *Journal of Agronomy and Crop Science*, *168*(3), 159-168. https://doi.org/10.1111/j.1439-037X.1992.tb00994.x

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed research*, *14*(6), 415-421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zeleke, K. T. (2019). Effect of grazing time and intensity on growth and yield of spring wheat (Triticum aestivum L.). *Journal of Integrative Agriculture*, *18*(5), 1138-1147. https://doi.org/10.1016/S2095-3119(18)62125-2

Zentella, R., Zhang, Z. L., Park, M., Thomas, S. G., Endo, A., Murase, K.,…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

Zhang, K., Zhang, J., Cui, C., Chai, L., Zheng, B., Jiang, L., & Li, H. (2025). The WRKY28-BRC1 Transcription Factor Module Controls Shoot Branching in Brassica napus. *Plants*, *14*(3), 486. https://doi.org/10.3390/plants14030486

Zhang, H. P., Turner, N. C., & Poole, M. L. (2010). Source-sink balance and manipulating sink-source relations of wheat indicate that the yield potential of wheat is sink-limited in high-

rainfall zones. *Crop & Pasture Science*, *61*(10), 852-861. https://doi.org/10.1071/CP10161

Zhang, J. Y., Tang, Y. Y., Pu, X., Qiu, X. B., Wang, J. H., Li, T., . . . Long, H. (2022). Genetic and transcriptomic dissection of an artificially induced paired spikelets mutant of wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *135*(7), 2543-2554. https://doi.org/10.1007/s00122-022-04137-5

Zhang, L. C., He, G. H., Li, Y. P., Yang, Z. Y., Liu, T. Q., Xie, X. Z., . . . Sun, J. Q. (2022). PIL transcription factors directly interact with SPLs and repress tillering/branching in plants. *New Phytologist*, *233*(3), 1414-1425. https://doi.org/10.1111/nph.17872

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

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

Zhou, Y., Liu, J., Guo, J., Wang, Y., Ji, H., Chu, X.,…Li, H. (2022). GmTDN1 improves wheat yields by inducing dual tolerance to both drought and low-N stress. *Plant Biotechnology Journal*, *20*(8), 1606-1621. https://doi.org/10.1111/pbi.13836

Zhu, Y. H., Ren, L. L., Horton, R., Lu, H. S., Wang, Z. L., & Yuan, F. (2018). Estimating the Contribution of Groundwater to the Root Zone of Winter Wheat Using Root Density Distribution Functions. *Vadose Zone Journal*, *17*(1). https://doi.org/10.2136/vzj2017.04.0075

Zimmerl, S., Lafferty, J., & Buerstmayr, H. (2014). Assessing diversity in T riticum durum cultivars and breeding lines for high versus low cadmium content in seeds using the CAPS marker usw47. *Plant Breeding*, *133*(6), 712-717. https://doi.org/10.1111/pbr.12218

Zwirek, M., Waugh, R., & McKim, S. M. (2019). Interaction between row-type genes in barley controls meristem determinacy and reveals novel routes to improved grain. *New Phytol*, *221*(4), 1950-1965. https://doi.org/10.1111/nph.15548

APPENDICES

APPENDIX A

CHAPTER 2 SUPPLEMENTARY INFORMATION

Table A2.1. Primer list: this table contains sequences and descriptions of all oligonucleotides used in PCR amplification and sequencing.

Gene	Description	ID	Primer Sequence (5'-3')	Source
GNI-A1	External Forward Primer	GNI-A1F	AGTCTCCAAAATTAAGTGGCAT	Sakuma *et al.* 2019
GNI-A1	External Reverse Primer	GNI-A1R	TGCCATTAATACACACTCTCCA	Sakuma *et al.* 2019
GNI-A1	Promoter Region Forward Primer	GNI-A1EPF	CCTGGCTCGGAAAGCACCTA	Sakuma *et al.* 2019
GNI-A1	Exon 2 Forward Primer	GNI-A1E2F	GGATGGTAACGGCTGGGAGA	Sakuma *et al.* 2019
GNI-A1	Exon 3 Reverse Primer	GNI-A1E3R	GTCGCTCTCAGCTTCTCCTT	Sakuma *et al.* 2019
GNI-D1	External Forward Primer	GNI-DexF	TTCGCCTAAAAGCGCCTTTG	This paper
GNI-D1	External Reverse Primer	GNI-DexR	CGAGAGTGTTGATTAATGGCAA	This paper

Table A2.2. GNI Variety Screen: This table contains a list of all varieties screened for *GNI-A1* polymorphism along with supplemental information for each variety.

Variety	*GNI-A1*	Class	USDA #, Canada #	Origin	Release Year
Carpio	*105N*	Durum	PI 670039	North Dakota, USA	2012
MT Blackbeard	*105N*	Durum	Pending	Montana, USA	2022
Alzada	*105Y*	Durum	PI 634820	WestBred LLC, USA	2004
CDC Precision	*105Y*	Durum	7832	Saskatchewan, Canada	2015
CDC Vivid	*105Y*	Durum	7220	Saskatchewan, Canada	2012
Divide	*105Y*	Durum	PI 642021	North Dakota, USA	2005
Joppa	*105Y*	Durum	PI 673106	North Dakota, USA	2014
Mountrail	*105Y*	Durum	PI 607530	North Dakota, NDSU Research Fdn., USA	1999
MT Raska	*105Y*	Durum	Pending	Montana, USA	2022
Lustre	*105Y*	Durum	PI 695072	Montana, USA	2020
ND Riveland	*105Y*	Durum	PI 687796	North Dakota Res. Foundation, USA	2018
Tioga	*105Y*	Durum	PI 660664 PVPO	North Dakota, NDSU Research Fdn., USA	2011
Alum	*105N*	HRS	PI 676289	Washington, USA	2015

Choteau	*105N*	HRS	PI 633974	Montana, USA	2003
Corbin	*105N*	HRS	PI 648028 PVPO	WestBred LLC, Bozeman, MT, USA	2006
Lanning	*105N*	HRS	PI 676978	Montana, USA	2016
Dagmar	*105K*	HRS	PI 690450	Montana, USA	2019
NS Presser CLP	*105K*	HRS	PI 679964	Montana: Northern Seeds, LLC Bozeman, MT, USA	2016
Reeder	*105K*	HRS	PI 613586	North Dakota, NDSU Research Fdn., USA	1999
Vida	*105K*	HRS	PI 642366	Montana, USA	2006
Brennan	*105Y*	HRS	PI 658041 PVPO	Syngenta Seeds, Inc., Junction City. KS, USA	2009
Chinese Spring	*105Y*	HRS	CItr14108	China	1932
Egan	*105Y*	HRS	PI 671855	Montana, USA	2014
Hi-Line	*105Y*	HRS	PI 549275	Montana, USA	1992
McNeal	*105Y*	HRS	PI 574642	Montana, USA	1994
SY Ingmar	*105Y*	HRS	PI 672586	Syngenta Seeds, Inc., USA	2014
Sy Soren	*105Y*	HRS	PI 662048	AgriPro, Syngenta, USA	2011
Sy Valda	*105Y*	HRS	PI 674341	Syngenta Seeds, Inc., USA	2015
WB-Gunnison	*105Y*	HRS	PI 665064	WestBred-Monsanto Bozeman, MT, USA	2011
Decade	*105N*	HRW	PI 660291	Montana, North Dakota, USA	2010
Flathead	*105N*	HRW	PI 693327	Montana, USA	2019
FourOsix	*105N*	HRW	PI 689753	Montana, USA	2018
Judee	*105N*	HRW	PI 665227	Montana, USA	2011
Loma	*105N*	HRW	PI 680576	Montana, USA	2016
MTF1435 (Stormbreaker for AgWest)	*105N*	HRW (forage)	PI 689773	Montana: AgWest Seeds, Ephraim, UT; Sioux Nation Ag, Fort Pierre, SD, USA	2018
Northern	*105N*	HRW	PI 676026	Montana, USA	2015
Ray	*105N*	HRW (forage)	PI 689754	Montana, USA	2018
SY Clearstone 2CL	*105N*	HRW (CL2)	PI 668090	Montana, Syngenta Seeds, Inc., USA	2012
Warhorse	*105N*	HRW	PI 670157	Montana, USA	2013
Yellowstone	*105N*	HRW	PI 643428	Montana, USA	2005
Bearpaw	*105Y*	HRW	PI 665228	Montana, USA	2011
Bobcat	*105Y*	HRW	PI 693325	Montana, USA	2019
Rampart	*105Y*	HRW	PI593889	Montana, USA	1996
StandClear CLP	*105Y*	HRW	PI 693326	Montana: Nutrien-Loveland Products, Inc. Loveland, CO, USA	2020

Table A2.3. GNI RIL Data: This table contains least squared means split into different environments and years for all parameters measured in the RIL population.

Environment	*GNI-A1*	N[a]	Height cm	Flag Leaf Length cm	Flag Leaf Width cm	Yield Kg/Ha
Rainfed 2021	*105N*	76	86.9±0.8	14.9±0.6	1.29±0.03	6423±153
	105K	66	88.1±0.9	15.6±0.6	1.29±0.04	6566±156
Rainfed 2022	*105N*	76	84.1±0.8	18.8±0.6	1.44±0.03	3143±153
	105K	66	85.8±0.9	19.0±0.6	1.41±0.04	3234±156
Irrigated 2021	*105N*	76	89.2±0.8	15.9±0.6	1.38±0.03	***8009±153***
	105K	66	88.9±0.9	16.7±0.6	1.37±0.04	***7669±156***
Irrigated 2022	*105N*	76	91.5±0.8	***18.3±0.6***[b]	1.41±0.03	7278±153
	105K	66	93.1±0.9	***19.4±0.6***	1.41±0.04	7401±156

Environment	*GNI-A1*	N[a]	Protein Content Percent	Bulk SGW mg	Spike Length cm	Fertile Spikelets No./Spike
Rainfed 2021	*105N*	76	13.7±0.1	***30.3±0.4***	8.74±0.2	17.1±0.2
	105K	66	13.6±0.1	***29.4±0.4***	8.87±0.2	17.2±0.2
Rainfed 2022	*105N*	76	***15.1±0.1***	25.6±0.4	8.71±0.2	16.5±0.2
	105K	66	***14.8±0.1***	25.0±0.4	8.86±0.2	16.7±0.2
Irrigated 2021	*105N*	76	13.4±0.1	33.6±0.4	9.52±0.2	17.3±0.2
	105K	66	13.4±0.1	33.1±0.4	9.77±0.2	17.4±0.2
Irrigated 2022	*105N*	76	13.4±0.1	31.9±0.4	8.56±0.2	16.5±0.2
	105K	66	13.3±0.1	31.9±0.4	8.57±0.2	16.5±0.2

Environment	*GNI-A1*	N[a]	Grains/ Spikelet No./Spikelet	Primary Spike SGW mg	Grains/ Primary Spike No./Spike	Yield/ Primary Spike g
Rainfed 2021	*105N*	76	***2.80±0.03***	***33.3±0.4***	***48.0±0.7***	1.59±0.03
	105K	66	***2.92±0.03***	***32.0±0.5***	***50.3±0.7***	1.60±0.03
Rainfed 2022	*105N*	76	***2.72±0.03***	28.7±0.4	***44.9±0.7***	***1.28±0.03***
	105K	66	***2.88±0.03***	28.0±0.5	***48.1±0.7***	***1.34±0.03***
Irrigated 2021	*105N*	76	***2.71±0.03***	***37.5±0.4***	***46.9±0.7***	***1.75±0.03***
	105K	66	***2.91±0.03***	***36.0±0.5***	***50.7±0.7***	***1.81±0.03***
Irrigated 2022	*105N*	76	***2.69±0.03***	34.6±0.4	***44.5±0.7***	***1.53±0.03***
	105K	66	***2.81±0.03***	34.6±0.5	***46.4±0.7***	***1.60±0.03***

[a] Denotes the number of lines in each genotype class.
[b] Bolding and Italics Denotes genotype classes that are significantly different ($p < 0.05$) within a single environment

Table A2.4, *GNI-A1* NIL Data: This table contains least squared means split into different environments for all parameters measured in the NIL populations.

Background	**Environment**	***GNI-A1***	N[a]	**Height** cm	**Flag Leaf Length** cm	**Flag Leaf Width** cm	**Yield** Kg/Ha
Vida/Spring Yellowstone	Irrigated 2022	*105N*	6	***74.9±0.6***[b]	16.4±0.5	1.27±0.03	1205±72
		105K	6	***77.3±0.6***	17.2±0.5	1.26±0.03	1277±69
	Rainfed 2022	*105N*	6	***89.9±0.5***	18.8±0.4	1.32±0.02	2113±61
		105K	6	***92.1±0.5***	19.9±0.4	1.31±0.02	2100±61
Lanning/ Egan	Irrigated 2022	*105N*	10	75.8±0.5	15.8±0.4	1.30±0.02	1302±41
		105Y	10	76.7±0.5	16.3±0.4	1.34±0.02	1337±42
	Rainfed 2022	*105N*	10	88.3±0.4	20.8±0.4	1.4±0.02	2261±38
		105Y	10	88.6±0.4	20.1±0.4	1.41±0.02	2278±41

Background	**Environment**	***GNI-A1***	N[a]	**Grain Protein Content** Percent	**Bulk SGW** mg	**Spike Length** cm	**Fertile Spikelets** No./spike
Vida/Spring Yellowstone	Irrigated 2022	*105N*	6	17.2±0.1	28±0.7	7.53±0.2	13.5±0.2
		105K	6	17.0±0.1	28.1±0.7	7.69±0.1	13.9±0.2
	Rainfed 2022	*105N*	6	16.1±0.1	31.9±0.7	7.62±0.1	13.6±0.2
		105K	6	16.0±0.1	31.4±0.7	7.59±0.1	13.8±0.2
Lanning/ Egan	Irrigated 2022	*105N*	10	17.3±0.1	27.8±0.4	8.51±0.2	14.3±0.2
		105Y	10	17.1±0.1	28.0±0.5	8.59±0.2	14.5±0.2
	Rainfed 2022	*105N*	10	16.6±0.1	33.4±0.4	8.29±0.2	14.7±0.2
		105Y	10	16.4±0.1	32.4±0.4	8.43±0.2	14.6±0.2

Background	**Environment**	***GNI-A1***	N[a]	**Grains/ Spikelet** No./Spikelet	**Primary Spike SGW** cm	**Grains/ Primary Spike** No./Spike	**Yield/ Primary Spike** g
Vida/Spring Yellowstone	Irrigated 2022	*105N*	6	***2.57±0.05***	***29.3±0.5***	***34.8±1.0***	1.02±0.04
		105K	6	***2.71±0.04***	***27.7±0.4***	***37.8±1.0***	1.05±0.04
	Rainfed 2022	*105N*	6	***2.49±0.03***	31.8±0.4	34.0±0.8	1.08±0.03
		105K	6	***2.61±0.04***	32.0±0.4	36.0±0.8	1.15±0.03
Lanning/ Egan	Irrigated 2022	*105N*	10	***2.97±0.05***	28.7±0.5	***42.5±0.9***	1.22±0.03
		105Y	10	***3.17±0.05***	28.2±0.5	***45.8±0.9***	1.29±0.03
	Rainfed 2022	*105N*	10	2.84±0.04	34.1±0.4	41.6±0.8	1.42±0.03
		105Y	10	2.95±0.05	33.4±0.5	43.2±0.9	1.44±0.03

Background	Environment	*GNI-A1*	N[a]	Milling Yield Percent	Flour Protein Percent	Flour Ash Percent
Vida/Spring Yellowstone	Irrigated 2022	*105N*	6	67.5±0.4	15.6±0.1	50.1±0.6
		105K	6	68.5±0.4	15.4±0.1	50.7±0.6
	Rainfed 2022	*105N*	6	***68.7±0.3***	14.7±0.1	49.7±0.4
		105K	6	***70.0±0.3***	14.6±0.1	49.7±0.4
Lanning/ Egan	Irrigated 2022	*105N*	10	***69.41±0.2***	15.9±0.1	47.5±0.5
		105Y	10	***68.85±0.2***	15.7±0.1	48.1±0.5
	Rainfed 2022	*105N*	10	***70.17±0.1***	15.0±0.1	47.3±0.5
		105Y	10	***69.76±0.1***	15.0±0.1	47.5±0.5

Background	Environment	*GNI-A1*	N[a]	Bran Percent	Kernel Diameter mm	Kernel Hardness KHI
Vida/Spring Yellowstone	Irrigated 2022	*105N*	6	28.4±0.3	2.55±0.04	80.8±1.7
		105K	6	27.6±0.3	2.56±0.04	78.1±1.6
	Rainfed 2022	*105N*	6	27.0±0.3	2.76±0.03	77.2±1.4
		105K	6	26.3±0.3	2.68±0.03	76.8±1.4
Lanning/ Egan	Irrigated 2022	*105N*	10	***26.9±0.2***	2.56±0.02	81.6±0.9
		105Y	10	***27.4±0.1***	2.58±0.02	82.4±0.9
	Rainfed 2022	*105N*	10	26.2±0.1	2.79±0.02	79.9±0.8
		105Y	10	26.5±0.1	2.77±0.02	82.1±0.9

[a] Denotes the number of lines in each genotype class.

[b] Bolding with Italics Denotes genotype classes that are significantly different ($p < 0.05$) within a single environment

APPENDIX B

CHAPTER 4 SUPPLEMENTARY INFORMATION

Table B4.1 Durum wheat *Rht-1* NIL experiment data broken down by environment and year

The impact of *Rht-1* durum wheat mutations on Plant Height across 5 Locations

Tested Allele	Background	Genotype	Bozeman 2022 Rainfed	Bozeman 2022 Irrigated	Bozeman 2023 Rainfed	Havre 2023 Rainfed	Sidney 2023 Rainfed
			cm				
Rht-B1b-E529K	*Rht-B1a* (Lustre)	mutant	70.8±2.0***	87.3±2.0***	95.5±2.0	77.1±2.4	83.9±2.4
		wildtype	75.9±2.1	95.1±2.1	99.6±2.0	81.0±2.4	87.2±2.4
	Rht-B1b (MT112219)	mutant	77.4±2.4***	88.2±2.4***	97.7±2.5***	87.7±2.9***	90.7±2.9***
		wildtype	61.0±2.1	70.7±2.1	74.9±2.3	74.7±2.7	72.9±2.7
Rht-A1-L358F	*Rht-B1a* (Lustre)	mutant	76.9±1.3	94.6±1.3	104.4±1.4	83.1±1.8	91.2±1.8
		wildtype	78.3±1.3	95.6±1.3	105.4±1.4	85.5±1.8	92.2±1.8
	Rht-B1b (MT112219)	mutant	56.6±0.8	62.9±0.8	70.0±1.1	69.4±1.3	67.0±1.3
		wildtype	56.1±0.9	63.1±0.9	68.4±1.1	69.0±1.3	65.1±1.3
Rht-A1-S50F	*Rht-B1a* (Lustre)	mutant	76.5±0.7*	93.3±0.7**	105.9±1.0	82.2±1.4	92.5±1.4
		wildtype	78.6±0.7	96.1±0.7	106.8±1.0	85.4±1.4	94.8±1.4
	Rht-B1b (MT112219)	mutant	57.9±0.7	66.3±0.8	70.2±1.1	71.2±1.3	70.1±1.3
		wildtype	56.1±0.7	64.4±0.7	68.6±1.1	69.9±1.3	67.8±1.3

Pairwise comparisons, *Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level

The impact of *Rht-1* durum wheat mutations on Grain Yield across 5 Locations

Tested Allele	Background	Genotype	Bozeman 2022 Rainfed	Bozeman 2022 Irrigated	Bozeman 2023 Rainfed	Havre 2023 Rainfed	Sidney 2023 Rainfed
			kg Ha^{-1}				
Rht-B1b-E529K	*Rht-B1a* (Lustre)	mut	2953±500	6089±500	5893±374	1991±526	4917±526
		wt	2963±504	6126±504	5826±373	2030±526	4904±526
	Rht-B1b (MT112219)	mut	2441±163	4824±163	6757±197*	2492±244	3911±244
		wt	2629±148	5066±148	6210±195	2351±242	4255±242
Rht-A1-L358F	*Rht-B1a* (Lustre)	mut	2818±115	6090±115	6472±168	2195±224	4661±224
		wt	2901±115	5972±115	5956±168	2197±224	4659±224
	Rht-B1b (MT112219)	mut	2818±215	5121±215	6274±235	2429±304	4290±304
		wt	2859±238	5144±235	6472±234	2573±303	4435±303
Rht-A1-S50F	*Rht-B1a* (Lustre)	mut	2727±308	5442±308	5964±259	2107±350	4454±350
		wt	2630±309	5588±309	5720±253	2097±350	4614±350
	Rht-B1b (MT112219)	mut	2424±222	4115±224*	6042±294	2232±333	4188±333
		wt	2629±214	4813±212	6388±292	2516±331	4376±331

Pairwise comparisons, *Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level

The impact of *Rht-1* durum wheat mutations on Single Grain Weight across 5 Locations

Tested Allele	Background	Genotype	Bozeman 2022 Rainfed	Bozeman 2022 Irrigated	Bozeman 2023 Rainfed	Havre 2023 Rainfed	Sidney 2023 Rainfed
			mg				
Rht-B1b-E529K	*Rht-B1a* (Lustre)	mut	33.3±1.8	38.8±1.8*	31.7±1.4	26.6±2.0	40.8±2.0
		wt	34.4±1.8	40.6±1.8	33.0±1.4	28.9±2.0	42.1±2.0
	Rht-B1b (MT112219)	mut	37.9±1.4	43.7±1.4*	41.9±1.3***	33.5±1.8	44.4±1.8*
		wt	37.3±1.4	41.6±1.4	34.1±1.3	30.2±1.8	40.1±2.0
Rht-A1-L358F	*Rht-B1a* (Lustre)	mut	35.4±1.1	41.5±1.1	35.1±1.4	32.7±1.7	42.0±1.7
		wt	36.6±1.1	42.2±1.1	33.2±1.5	32.4±1.7	41.5±1.7
	Rht-B1b (MT112219)	mut	34.9±1.7	41.5±1.7	35.9±1.4	29.5±1.9	41.3±1.9
		wt	35.0±1.7	41.1±1.7	34.1±1.4	29.6±1.9	40.6±1.9
Rht-A1-S50F	*Rht-B1a* (Lustre)	mut	35.8±1.6*	42.3±1.6	35.4±1.4	33.5±1.9	44.3±1.9
		wt	37.9±1.6	42.2±1.6	35.9±1.4	34.0±1.9	44.2±1.9
	Rht-B1b (MT112219)	mut	35.2±1.4	39.8±1.4	35.4±1.2	29.3±1.7	42.1±1.7
		wt	36.0±1.4	41.0±1.4	34.9±1.2	28.3±1.7	42.1±1.7

Pairwise comparisons, *Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level

The impact of *Rht-1* durum wheat mutations on Grain Protein Content across 5 Locations

Tested Allele	Background	Genotype	Bozeman 2022 Rainfed	Bozeman 2022 Irrigated	Bozeman 2023 Rainfed	Havre 2023 Rainfed	Sidney 2023 Rainfed
			g kg^{-1}				
Rht-B1b-E529K	*Rht-B1a* (Lustre)	mut	157±6.0	139±6.0	139±4.4	180±6.2	117±6.2*
		wt	158±6.0	142±6.0	143±4.4	181±6.2	112±6.2
	Rht-B1b (MT112219)	mut	157±4.6	147±4.6**	135±3.6	159±4.8	115±4.8
		wt	153±4.5	140±4.5	136±3.6	162±4.8	111±4.8
Rht-A1-L358F	*Rht-B1a* (Lustre)	mut	162±4.0	146±4.0	141±3.5	173±4.6	119±4.6
		wt	160±4.0	147±4.0	146±3.6	170±4.6	117±4.6
	Rht-B1b (MT112219)	mut	150±3.3	134±3.3	133±2.7	158±3.7	112±3.7
		wt	151±3.5	134±3.5	134±2.8	156±3.7	108±3.7
Rht-A1-S50F	*Rht-B1a* (Lustre)	mut	162±3.1	150±3.1	140±2.5*	174±3.5	113±3.5
		wt	164±3.1	151±3.1	145±2.5	175±3.5	113±3.5
	Rht-B1b (MT112219)	mut	157±4.3*	143±4.4	137±3.7	162±4.7	120±4.7*
		wt	151±4.3	139±4.3	131±3.6	158±4.7	111±4.7

Pairwise comparisons, *Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level

APPENDIX C

CHAPTER 5 SUPPLEMENTARY INFORMATION

Table C5.1. Grain Yield, *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele				kg ha^{-1}								
TB-A1a	*TB-B1a*	4104	2778	5514	5578	2165	3299	5156	3845	6128	3377	2159	5144
tb-A1-W339*	*TB-B1a*	4129	3234	5984	5661	2200	3184	5013	3968	6089	3225	1909	4956
TB-A1a	*tb-B1*-W341*	4154	3337	6361	5788	2162	3275	5128	3636	5871	3321	1897	4919
tb-A1-W339*	*tb-B1*-W341*	4033	2916	5825	5569	2169	2900	5145	3713	6145	3317	1731	4934
	$LSD_{0.05}$	**94.7**	**338**	**338**	239	338	338	338	338	338	275	**275**	275
P values	Genotype	0.0021											
	Environment	<0.0001											
	GXE	<0.0001											

Table C5.2 Mature Tillers, *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele				tillers m^{-2}								
TB-A1a	*TB-B1a*	601	533	760	911	379	385	706	595	607	414	632	684
tb-A1-W339*	*TB-B1a*	613	568	795	912	383	402	713	583	612	450	632	694
TB-A1a	*tb-B1*-W341*	631	553	892	978	388	435	685	560	658	445	684	663
tb-A1-W339*	*tb-B1*-W341*	657	639	893	940	460	436	692	669	643	470	683	701
	$LSD_{0.05}$	**21.6**	**77.2**	**77.2**	**54.5**	**77.2**	77.2	77.2	**77.2**	77.2	62.9	62.9	62.9
P values	Genotype	<0.0001											
	Environment	<0.0001											
	GXE	0.15											

Table C5.3. Productive Heads, *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele				heads m^{-2}								
TB-A1a	*TB-B1a*	542	496	615	814	324	362	646	562	587	408	503	646
tb-A1-W339*	*TB-B1a*	555	544	640	801	339	362	653	568	591	444	494	669
TB-A1a	*tb-B1*-W341*	566	525	720	878	326	404	597	540	622	440	544	627
tb-A1-W339*	*tb-B1*-W341*	586	591	694	848	366	388	641	635	618	452	544	674
	$LSD_{0.05}$	**19.3**	**68.8**	**68.8**	**48.7**	68.8	68.8	68.8	**68.8**	68.8	56.1	56.1	56.1
P values	Genotype	<0.0001											
	Environment	<0.0001											
	GXE	0.2											

Table C5.4. Plant Height, *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele				cm								
TB-A1a	*TB-B1a*	69.9	63.6	72.6	73.2	68.4	72.6	70	75.1	73.6	69.5	61.6	68.7
tb-A1-W339*	*TB-B1a*	67.1	63.2	69.9	70.5	66.1	69.9	67.1	71.2	69.4	66.8	58.4	65.5
TB-A1a	*tb-B1*-W341*	68.4	63.7	70.5	71.6	68.3	71.6	68	72.8	71.4	68	59.6	67.4
tb-A1-W339*	*tb-B1*-W341*	67.1	62.5	70.2	70.4	66.7	68.9	67	71.1	70.6	66.4	57.4	66.6
	$LSD_{0.05}$	**0.51**	1.8	**1.8**	**1.27**	1.8	**1.8**	**1.8**	**1.8**	**1.8**	**1.47**	**1.47**	**1.47**
P values	Genotype	<0.0001											
	Environment	<0.0001											
	GXE	0.66											

Table C5.5. Spikelets per Spike (Primary Head), *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele						no						
TB-A1a	*TB-B1a*	14.8	15.3	15.4	14.6	12.9	nd	14	16	16.1	14.7	14.1	15.2
tb-A1-W339*	*TB-B1a*	14.9	15.3	15.5	14.6	13	nd	14.1	16.2	16.1	14.8	13.8	15.2
TB-A1a	*tb-B1*-W341*	14.3	14.8	14.9	13.9	12.6	nd	13.5	15.2	15.5	14.3	13.4	14.8
tb-A1-W339*	*tb-B1*-W341*	14.2	14.8	14.5	13.8	12.3	nd	13.6	15.1	15.2	14.7	13.2	14.6
	$LSD_{0.05}$	**0.16**	0.56	**0.56**	**0.4**	0.56		0.56	**0.56**	**0.56**	0.46	**0.46**	0.46
P values	Genotype	<0.0001											
	Environment	<0.0001											
	GXE	0.729											

Table C5.6. Single Grain Weight (Bulk Sample), *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele						mg						
TB-A1a	*TB-B1a*	33.6	29.5	32.9	34.7	39.9	33.3	30.6	32.1	41.3	36.2	23.8	35.1
tb-A1-W339*	*TB-B1a*	33.5	29.6	34.7	34.3	39.9	34	29.5	33.2	40.9	35.4	21.8	35
TB-A1a	*tb-B1*-W341*	33.6	29.8	33.3	35.7	40.6	33.8	29.9	32.7	41.4	36	20.9	35.2
tb-A1-W339*	*tb-B1*-W341*	33.3	28.9	33	35.8	40.9	31.8	30.5	32.4	41.2	35.8	20.6	34.8
	$LSD_{0.05}$	0.5	1.8	1.8	1.27	1.8	1.8	1.8	1.8	1.8	1.47	**1.47**	1.47
P values	Genotype	0.504											
	Environment	<0.0001											
	GXE	0.01											

Table C5.7. Grain Yield per Primary Head, *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele						g						
TB-A1a	*TB-B1a*	1.51	1.56	1.85	1.61	1.45	nd	1.47	1.48	1.79	1.43	0.91	1.54
tb-A1-W339*	*TB-B1a*	1.44	1.4	1.74	1.54	1.37	nd	1.47	1.47	1.65	1.4	0.85	1.48
TB-A1a	*tb-B1*-W341*	1.43	1.47	1.73	1.5	1.33	nd	1.5	1.35	1.69	1.39	0.88	1.5
tb-A1-W339*	*tb-B1*-W341*	1.34	1.27	1.55	1.49	1.2	nd	1.42	1.29	1.66	1.36	0.8	1.38
	$LSD_{0.05}$	**0.033**	**0.114**	**0.114**	**0.08**	**0.114**		0.114	0.114	0.114	0.093	0.093	0.093
P values	Genotype	<0.0001											
	Environment	<0.0001											
	GXE	0.068											

Table C5.8. Seeds per Spikelet (Primary Head), *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele						no						
TB-A1a	*TB-B1a*	2.9	3.2	3.3	3.1	3.4	nd	2.6	2.6	2.6	2.6	2.5	2.8
tb-A1-W339*	*TB-B1a*	2.7	2.9	3.1	2.9	3.1	nd	2.6	2.5	2.5	2.5	2.5	2.6
TB-A1a	*tb-B1*-W341*	2.8	3	3.1	3	3.2	nd	2.6	2.5	2.5	2.6	2.6	2.7
tb-A1-W339*	*tb-B1*-W341*	2.7	2.8	3	2.9	3	nd	2.6	2.4	2.6	2.5	2.5	2.6
	$LSD_{0.05}$	**0.054**	0.19	0.19	0.13	0.19		0.19	0.19	0.19	0.15	0.15	0.15
P values	Genotype	<0.0001											
	Environment	<0.0001											
	GXE	0.142											

Table C5.9. Grain Protein Content, *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele				g kg^{-1}								
TB-A1a	*TB-B1a*	140	151	135	138	164	125	99	147	139	145	172	120
tb-A1-W339*	*TB-B1a*	143	152	134	141	167	127	102	152	141	149	184	123
TB-A1a	*tb-B1*-W341*	141	149	132	137	164	125	101	150	142	147	182	120
tb-A1-W339*	*tb-B1*-W341*	144	153	137	137	168	133	104	155	141	149	186	123
	$LSD_{0.05}$	**1.6**	5.8	5.8	4.1	5.8	**5.8**	5.8	5.8	5.8	4.7	**4.7**	4.7
P values	Genotype	<0.0001											
	Environment	<0.0001											
	GXE	0.033											

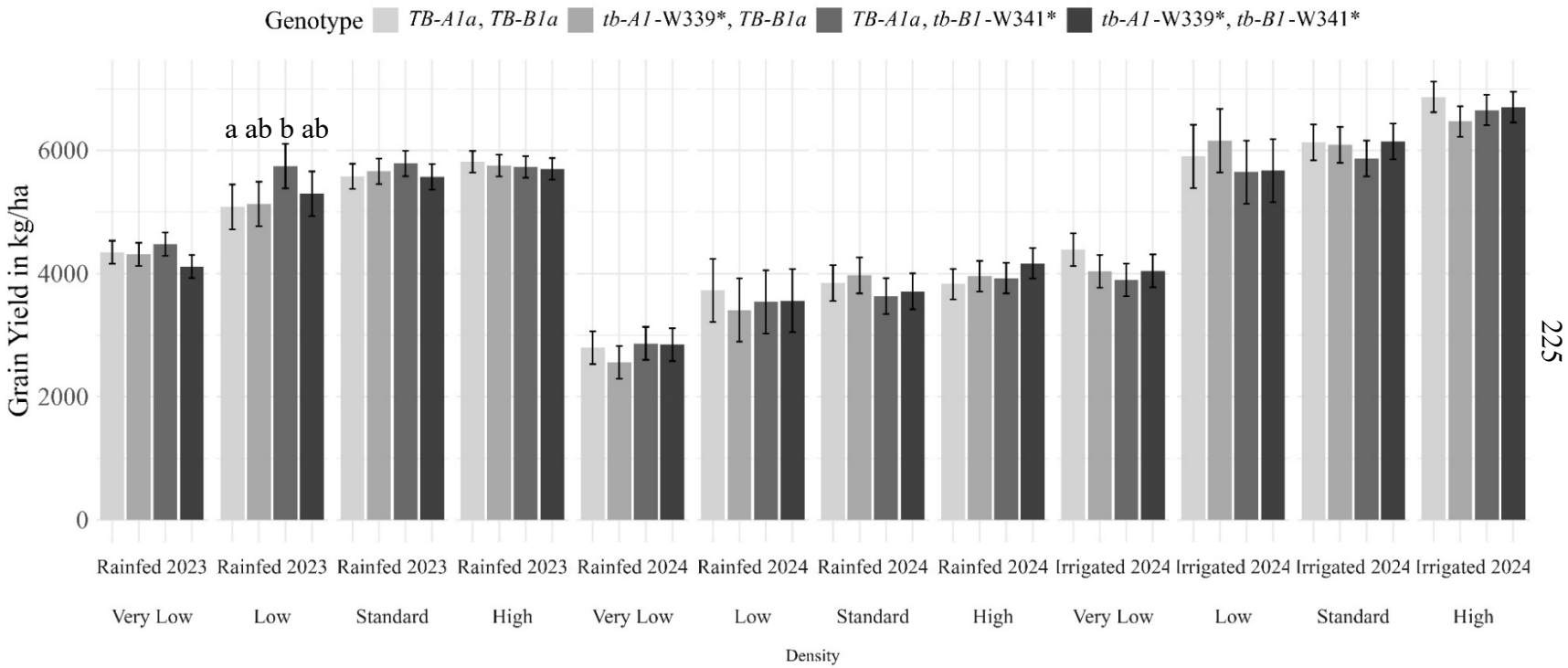
Table C5.10. Flag Leaf Length, *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023*	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele				cm								
TB-A1a	*TB-B1a*	21.8	20.6	22.1	28.2	nd	nd	nd	21.5	16.5	nd	nd	nd
tb-A1-W339*	*TB-B1a*	21.3	20.5	20.3	28.1	nd	nd	nd	20.4	17.1	nd	nd	nd
TB-A1a	*tb-B1*-W341*	21.7	21.1	21.6	27.6	nd	nd	nd	22.2	16.1	nd	nd	nd
tb-A1-W339*	*tb-B1*-W341*	20.9	19.6	19.2	27.7	nd	nd	nd	20.6	17.5	nd	nd	nd
	$LSD_{0.05}$	0.7	1.6	1.6	1.1				1.6	1.6			
P values	Genotype	0.07826											
	Environment	<0.0001											
	GXE	0.01377											

Table C5.11. Flag Leaf Width, *TB1* Durum NIL All Location/Years

Genotype		Overall Mean	Rainfed Bozeman 2022	Irrigated Bozeman 2022	Rainfed Bozeman 2023	Rainfed Havre 2023	Rainfed Moccasin 2023	Rainfed Sidney 2023	Rainfed Bozeman 2024	Irrigated Bozeman 2024	Rainfed Havre 2024	Rainfed Moccasin 2024	Rainfed Sidney 2024
TB-A1 Allele	*TB-B1* Allele				cm								
TB-A1a	*TB-B1a*	1.39	1.32	1.31	1.62	nd	nd	nd	1.33	1.35	nd	nd	nd
tb-A1-W339*	*TB-B1a*	1.4	1.38	1.32	1.64	nd	nd	nd	1.32	1.34	nd	nd	nd
TB-A1a	*tb-B1*-W341*	1.39	1.38	1.31	1.58	nd	nd	nd	1.32	1.36	nd	nd	nd
tb-A1-W339*	*tb-B1*-W341*	1.38	1.33	1.31	1.57	nd	nd	nd	1.32	1.36	nd	nd	nd
	$LSD_{0.05}$	0.04	0.09	0.09	0.07				0.09	0.09			
P values	Genotype	0.451											
	Environment	<0.0001											
	GXE	0.8383											

Supplementary Figure C5.1. Grain yield across different densities in 2023 and 2024 Bozeman environments. Letters indicate significant differences between genotypes ($p < 0.05$). In Bozeman seeding rate experiments, plots were planted at high seeding rate of 391 seeds m^{-2}, a standard seeding rate of 196 seeds m^{-2}, a low rate of 98 seeds m^{-2}, and a very low rate of 42 seeds m^{-2}



APPENDIX D

CHAPTER 6 SUPPLEMENTARY INFORMATION

Table D6.1. Vida by Spring Yellowstone RIL Population data split up by environment and year. Bold indicates significant pairwise comparison between genotypes within environment/year. Vida/Spring Yellowstone RILs, *TB-D1b* natural mutants (76 RILs) compared with *TB-D1a* wildtypes (65 RILs). Two years, two environments, two reps each. (Tiller parameters measured in only one replicate in Rainfed 2021, Rainfed 2022, and irrigated 2022 environments.)

Environment	***TB1* Genotype**	**Heading Date**	**Maturity Date**	**Tillers at Maturity**	**Productive Spikes**	**Leaf Length**	**Plant Height**
		d	d	tillers m^{-2}	spikes m^{-2}	cm	cm
Rainfed 2021	*TB-D1b*	179.3±0.3	215.0±0.3	738±52	646±65	15.5±0.62	87.0±0.8
	TB-D1a	179.5±0.3	214.4±0.3	721±53	633±65	15.1±0.63	87.9±0.8
Rainfed 2022	*TB-D1b*	193.0±0.3	222.3±0.3	701±52	620±65	19.0±0.62	84.5±0.8
	TB-D1a	193.3±0.3	222.2±0.3	709±53	621±65	18.9±0.63	85.0±0.8
Irrigated 2021	*TB-D1b*	180.2±0.3	221.1±0.3	868±37	740±46	16.5±0.62	88.7±0.8
	TB-D1a	180.4±0.3	221.3±0.3	874±37	743±46	16.1±0.63	89.4±0.8
Irrigated 2022	*TB-D1b*	194.4±0.3	231.4±0.3	912±52	818±65	18.9±0.62	92.0±0.8
	TB-D1a	194.7±0.3	231.4±0.3	910±53	802±65	18.9±0.63	92.6±0.8
ANOVA							
TB-D1		0.406	0.82	0.93	0.71	0.3845	0.4353
Env.		< 0.0001	< 0.0001	< 0.01	0.11	< 0.0001	< 0.0001
GxE		0.782	0.24	0.71	0.74	0.5302	0.7715

Environment	***TB1* Genotype**	**Grain Yield**	**Single Seed Wt**	**Grain Protein**	**Spikelets/ Spike**	**Yield/ Spike**	**Seeds/ Spikelet**
		kg ha^{-1}	mg	g kg^{-1}	no	g	no.
Rainfed 2021	*TB-D1b*	**6587*±144**	30.2±0.4	136±1.1	17.7±0.2	1.58±0.02	2.85±0.03
	TB-D1a	**6358±147**	29.6±0.4	137±1.1	17.8±0.2	1.60±0.02	2.89±0.03
Rainfed 2022	*TB-D1b*	**3302*±144**	25.5±0.4	149±1.1	17.2±0.2	1.35**±0.02	2.82±0.03
	TB-D1a	**3030±147**	24.9±0.4	151±1.1	17.0±0.2	1.27±0.02	2.78±0.03
Irrigated 2021	*TB-D1b*	7766±144	33.4±0.4	135±1.1	17.9±0.2	1.78±0.02	2.79±0.03
	TB-D1a	7958±147	33.2±0.4	134±1.1	18.0±0.2	1.77±0.02	2.81±0.0
Irrigated 2022	*TB-D1b*	7398±144	32.2±0.4	134±1.1	17.1±0.2	1.55±0.02	2.75±0.03
	TB-D1a	7231±147	31.4±0.4	134±1.1	17.2±0.2	1.57±0.02	2.74±0.03
ANOVA							
TB-D1		0.03	0.17	0.87	0.95	0.457324	0.94
Env.		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001
GxE		<0.001	0.19	0.02	0.23	0.0014	0.021

APPENDIX E

INTROGRESSING MUTANT ALLELES OF *TEOSINTE BRANCHED-1* INTO TRITICALE (*TRITICOSECALE* SPP.)— LAB AND GREENHOUSE METHODS

Abstract

Triticale *(Triticosecale* spp.*)* is a hybrid cereal species developed through interspecific crosses between wheat *(Triticum* spp.*)* and rye (*Secale cereale* L.)*,* initially reported as a sterile hybrid in 1875 and later rendered fertile through colchicine-induced chromosome doubling. While triticale retains the resilience and disease resistance of rye, its low grain quality has limited its widespread adoption as a food crop. In North America, triticale is primarily utilized as a forage crop, oftentimes outperforming other annual and perennial forage options in biomass production, particularly in the Northern Great Plains. However, triticale typically exhibits reduced tillering relative to wheat, a trait inherited from its rye ancestry. Given the established role of *Teosinte Branched-1 (TB1)* in suppressing tillering in grasses, targeted modification of *TB1* homologs in triticale may enhance tiller production and, consequently, forage yield. In this study, an ethyl methanesulfonate (EMS) induced mutagenesis approach was used to generate a novel mutant allele *tb-R1*-S184F in the R genome of forage triticale variety 'FX1001'. Additionally, the previously identified *tb-A1-R256** mutant allele was introgressed into triticale from winter wheat. These mutations have been combined in three different backgrounds in order to develop double *TB1* mutant triticale lines. PCR assays and a cleaved amplified polymorphic sequences (CAPS) marker were developed to genotype for the *tb-R1*-S184F allele. Both mutant alleles are being backcrossed to recurrent parents to generate near-isogenic lines (NIL) for field evaluation of their effects on tillering and biomass accumulation. This research provides a foundation for improving tillering capacity in forage triticale, with the goal of enhancing its agronomic performance.

Introduction

Triticale (*Triticosecale* spp.) was first described in 1875 as a vigorous but sterile F_1 hybrid between hexaploid wheat (*Triticum aestivum* L.) and Rye (*Secale cerale* L.) (Wilson, 1875). In the 1930's, usage of colchicine to induce chromosome doubling in plants was discovered. This made creating new fertile polyploid hybrids possible by doubling the chromosomes of sterile haploid hybrids (Blakeslee & Avery, 1937). Using these methods, fertile and vigorous triticale lines were developed, representing the first commonly grown, synthetically created, hybrid crop species (Lukaszewski & Gustafson, 2011). Triticale by definition is a hybrid of wheat and rye that can contain various combinations of different wheat (AABB or AABBDD) and rye (RR) genomes at different ploidy levels. Hexaploid (AABBRR) triticale is the most common in modern programs, with various octoploid and tetraploid varieties often used for novel trait introgression (Lelley, 2006; Lukaszewski & Gustafson, 2011). Additionally, crosses between hexaploid wheat and hexaploid triticale can produce viable seed when triticale is used as the female parent (Hills et al., 2007). Primary goals in triticale development have been to retain rye background traits that make it resistant to disease and harsh environmental conditions, but also to gain improved yield and quality traits from wheat. Approximately 90 % of the world's triticale acreage is grown in Europe for livestock feed grain and forage, and as biofuel. Triticale acreage in North America has increased since the 1970's, where it is generally grown as a forage crop (Baron et al., 2015; Mergoum et al., 2009). Triticale has been investigated as a substitute for wheat in making various products suitable for human consumption. However, flour produced from triticale grain is characterized by high dough stickiness, low gluten strength, low falling number, and low water absorption. These undesireable factors limit its use as a realistic human

food source, resulting in the vast majority of triticale research and development in the United States to be focused on forage production (McGoverin et al., 2011).

Forage triticale varieties (along with similarly adapted forage winter wheat lines) are commonly grown in the Northern Great Plains region as annual crops that provide a high yielding, high quality source of silage and hay during early summer months when annuals such as oats, barley, field pea, and alfalfa may be in short supply. Although spring habit varieties and semidwarf varieties exist, most forage varieties are winter habit with a tall phenotype. In Montana, these lines take advantage of early season moisture, and their height allows them to produce relatively high biomass yields. Overall, triticale forage lines tend to yield higher than forage winter wheat lines and other annual forage crops in this region (Lukaszewski & Gustafson, 2011; McVay et al., 2019).

Although triticale forage lines generally have higher forage yield potential than winter wheat lines, they tend to produce less tillers. This is a trait inherited from rye, which tillers significantly less than its wheat and barley relatives in both irrigated and non-irrigated environments. This makes triticale a prime target for tiller number improvement (Chaturvedi et al., 1981). *Teosinte Branched-1 (TB1)* was first characterized in maize *(Zea mays* L. subsp. *mays)* as a key determinant in the domestication of modern corn from its ancestral teosinte *(Zea mays* L. subsp. *parviglumis)*. It is a regulator of branching in plants and is part of the shade response pathway. Decreased expression of *TB1* is associated with increased tillering in grasses (Doebley et al., 1995; Doebley et al., 1997; Whipple et al., 2011). Additionally, mutations in homoeologs of *TB1* have been shown to increase tiller number in hexaploid and tetraploid wheat (Dixon et al., 2018; Volkman et al., 2022). Given the relatively low tillering potential of triticale,

introgressing mutant alleles of *TB1* into forage lines is a promising approach towards increasing tiller number and improving forage biomass yield.

In this study, *TB-R1* mutations were successfully generated through creation of an ethyl methanesulfonate (EMS) mutagenesis population in the triticale variety 'FX1001'. Additionally, a *TB-A1* mutant allele derived from hexaploid wheat was successfully backcrossed into a triticale background. These two alleles have been crossed together to generate double *TB1* mutant triticale lines in three different backgrounds. These lines will be further backcrossed to create near isogenic lines to test the efficacy of these alleles in increasing tiller number and biomass in triticale.

Materials, Methods, Continuing Results

Common triticale varieties are generally hexaploid, containing the A and B genome from wheat and the R genome from rye. Both tall and semidwarf varieties are grown in the United States, with the semidwarf varieties commonly containing the *Rht-B1b* semidwarfing allele from hexaploid wheat, which is closely linked to *TB-B1* on chromosome 4B. To create a triticale line with partially reduced function of *TB1* that could be easily crossed into both semidwarf and tall varieties containing different *Rht-B1* alleles, *TB-A1* and *TB-R1* were chosen for modification. To generate a novel *TB-R1* mutant allele, an EMS mutagenesis population was developed using the variety FX1001 and was screened for mutations. The *tb-A1*-R256* nonsense mutant allele originating from the 'Cadenza' TILLING population line 'WCAD-667' was chosen to be introgressed into the variety FX1001 through interspecific crossing with a forage winter wheat line containing the mutant allele (Krasileva et al., 2017).

Triticale EMS population development

The following methods were used to create an EMS mutagenesis population in triticale. Forty racks of 200 small conetainers each, 8000 total, were filled with Sunshine® mix soil (Sun Gro Horticulture, Agawam, MA, USA). Initially, seed soaked in 1 % EMS solution for 16 hours was planted. However, only around 60 (less than 1 %) of the plants sprouted, and about half of sprouted plants had no meristem, only a preformed cotyledon. Two additional populations of seeds were prepared using a 0.5 % EMS solution and 0.75 % EMS solution, and 3800 seeds were planted of each. Around 1300 or 34 % of seeds from the 0.5% EMS solution germinated, and around 550 or 14 % of the seeds from the 0.75 % EMS solution germinated. DNA was extracted from all 550 seedlings from the 0.75% solution and from 700 of the seedlings from the 0.5% solution. DNA samples and M_1 seed of these lines were catalogued for future use. The *TB-A1* gene and the *TB-R1* gene in each of the M_1 plant DNA samples was amplified by PCR and sequenced via sanger sequencing. To search for mutations in these genes of interest, M_1 sequence was aligned and analyzed against sequences of *TB-A1* and *TB-R1* amplified from the FX1001 parent line. Alignment and analysis was performed using SeqMan Pro® Version 17 (DNASTAR, Madison, WI). Eight M_2 seeds were planted representing each line that appeared to have a deleterious mutation in *TB-A1* or *TB-R1*. DNA samples of each M_2 plant were extracted, and sequence was analyzed to confirm presence or absence of the mutation. Ultimately only one heritable deleterious mutation in *TB-R1*, and no heritable deleterious mutations in *TB-A1* were found. The *TB-R1* mutation was characterized as *tb-R1*-S184F. This mutation was found in a line labeled as 'TB1R.FX1001.EMS15'. The *tb-R1*-S184F allele is the result of a missense mutation at the 184th codon of TCC to TTC with the C-T base pair change occurring at the 551 base pair

relative to the start codon. This mutation was determined to have a PROVEAN score of -2.736 which is predicted to be deleterious (Choi & Chan, 2015).

TB-R1 PCR Development

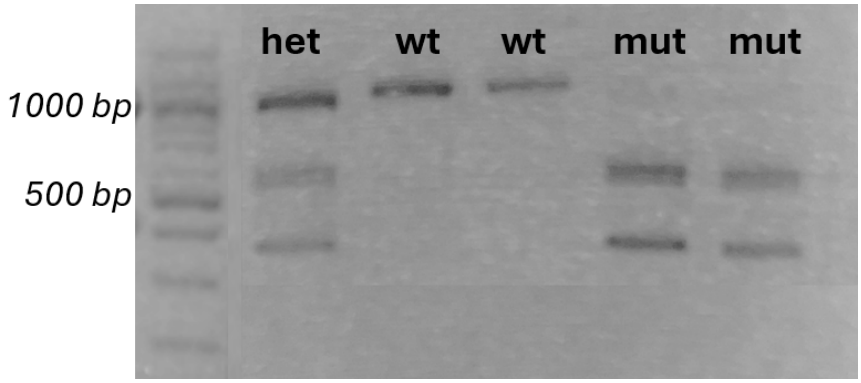
To develop the PCR protocol for the *TB-R1* gene in triticale for sequencing and genotyping, multiple forward and reverse primers, primer concentrations, and annealing temperatures were tested. Selected primers for amplification were *TB-R1*-For3 with the sequence 5'-CACAGACACAGTAGAAGCGC-3' and *TB-R1*-Rev2 with the sequence 5'-CTGCCGTACCCTCCG-3'. *TB-R1*-For3 binds to the promoter sequence, and *TB-R1*-Rev2 binds within the last 100 bp of the coding sequence, resulting in a 990 bp PCR product. Ideal PCR conditions for amplification of this gene is 38 cycles consisting of 30 seconds of denaturation at 96 °C, 30 seconds of annealing at 62 °C, and one minute of extension at 72 °C. Ideal reagent levels per single sample consist of 14.27 µl of ultrapure nuclease free water, 5 µl of 5x Green GoTaq® Flexi Buffer (Promega, Madison, WI), 2 µl of $MgCl_2$, 2 µl of dNTP, 0.2 µl of 20 mM Forward primer, 0.2 µl of 20 mM reverse primer, and 0.13 µl of µl GoTaq® G2 DNA Polymerase (Promega, Madison, WI).

Marker Development

An assay utilizing an allele specific qPCR PACE® genotyping marker (3CR Bioscience, UK) described in Chapter 6 was used to genotype for presence of the *tb-A1-W339** allele (Caleb Hale Thesis, Chapter 6). A cleaved amplified polymorphic sequences (CAPS) assay was developed to genotype for the *tb-R1*-S184F allele as follows. First, a standard PCR was performed using the above protocol with primers *TB-R1*-For3, and *TB-R1*-Rev2. The restriction enzyme EarI (New England Biolabs, Inc. Ipswich, MA) was used to digest PCR product. For

each individual sample, 10 µl of PCR product was digested with 0.1 µl of EarI restriction enzyme, 2 µl of CutSmart® Buffer (New England Biolabs, Inc. Ipswich, MA), and 7.9 µl of ultrapure nuclease free water. This digestion was incubated at 37 °C for two hours. After the digestion, samples were run on a 200 ml 1.5 % agarose gel at 180 volts for 45 minutes. This enzyme digest makes a single cut at the site of the *tb-R1*-S184F mutation, such that a digested homozygous mutant PCR product displays two bands when ran on an electrophoresis gel, one at 422 bp, and one at 570 base pairs. Homozygous wildtype samples only display the 990 bp band, and PCR products representing a heterozygous sample display all three bands (see Figure 1).

Figure E1. Gel image of the CAPS assay used to genotype for the *tb-R1*-S184F allele. Gel image shows from left to right one heterozygous sample, two wildtype homozygous *TB-R1a* samples, and two mutant homozygous *tb-R1*-S184F samples.



Experimental Line Development

To introgress the characterized *tb-R1*-S184F mutation into a background with less off target EMS derived mutations, M_3 seeds from the line TB1R.FX1001.EMS15 were planted and genotyped to ensure homozygosity for the *tb-R1*-S184F allele. These lines were crossed to the FX1001 parent line. Subsequent backcrosses were made to the FX1001 parent line. At each step, F_1 plants were genotyped to ensure heterozygosity for the mutant allele.

To introgress the *tb-A1-W339** allele from winter wheat into triticale, initial crosses were made between FX1001 and a 'Ray' (PI 689754) background BC_4F_2 line fixed for the *tb-A1-W339** mutant allele (Bruckner et al., 2019). Reciprocal interspecific crosses crosses were made between these two lines. When the winter wheat parent was used as the female, F_1 seeds were generated, but none of these F_1 seeds were viable when planted in the greenhouse. When the triticale parent was used as the female in these interspecific crosses, there were fewer F_1 seeds generated, but approximately 5 % of these seeds germinated and grew into full size, viable plants. The F_1 plants resulting from this cross were almost totally male sterile. Anthers produced on these plants appeared large, plump, and yellow, but upon closer inspection they did not produce pollen. Out of the eight F_1 plants grown to maturity, there were approximately 30 F_2 seeds generated as selfs, demonstrating the extremely low fertility rate of pollen produced from these plants. As such, spikes from these F_1 plants were not used as male pollen donors in subsequent crosses. For the first backcross to the triticale parent FX1001, male sterile F_1 plants were used as females, and the recurrent FX1001 parents were used as male pollen donors. BC_1F_1 seed resulting from these crosses was generated at a higher success rate than the initial F_1 cross, but resulting seeds were still shriveled, and again only 5 % of these seeds germinated and grew into a viable plant. These results are consistent with information provided in Hills et al. (2007).

In Fall 2023, two triticale lines were acquired from the University of Nebraska-Lincoln triticale breeding program for backcrossing, 'NT23226' and 'NT22705'. NT23226 is a forage/grain triticale with awnlettes and medium maturity. NT22705 is a forage/grain triticale with awnlettes and early maturity. FX1001 and NT22705 flower around the same time when planted in the greenhouse and NT23226 flowers four to six days later. Separate crosses between

these two triticale lines and with F_2 FX1001 background lines fixed for either the *tb-A1-W339** allele or the *tb-R1*-S184F allele were made in Spring 2024. Crosses between the resulting F_1 plants were made in Fall 2024 to combine the *tb-A1-W339** and *tb-R1*-S184F alleles in each of the three backgrounds. No plants were generated containing both alleles in the FX1001 background.

Germination Protocol

To provide seeds produced from interspecific crosses with the best possible chance for germination, harvested seeds were dried in a drying room at 43 °C for two days, and were then stored in a refrigerator at 2 °C for one week. Seeds were planted in moist soil in small conetainers, and then watered with a gibberellic acid solution at a concentration of 250 parts per million (90 µm). These planted seeds were subjected to a cold treatment at 4 °C in a vernalization chamber for two days. After this cold treatment, containers were placed in a greenhouse where temperatures were maintained at 22 °C during the day and 14 °C at night. A clear tub was placed over the tops of the conetainers as a cover to retain warmth and moisture to aid germination. Conetainers were not watered until they appeared dry, generally three to five days after the cold treatment. Once seedlings that had germinated, the clear cover was removed, and seedlings were continued to be watered with plain water. Once seedlings reached the two-leaf stage, they were subjected to eight weeks of vernalization in the cold room at 4 °C. Post vernalization, seedlings were transplanted to 20 cm diameter pots. Using these methods, the germination rate of F_1 seeds produced from interspecific crosses between triticale and winter wheat ranged from 5 % to 15 %. This germination rate increased with progressive backcrossing to the respective recurrent triticale parent.

Future crossing

Crosses between lines with the following pedigrees are to be made in March, 2025, with lines containing hexaploid wheat genetic material to be used as the female due to recurring male sterility in these lines. FX1001/tbA1.Ray//FX1001///FX1001*3/TB1R.FX1001.EMS15 will be crossed with FX1001*4/TBR1.FX1001.EMS15. FX1001/tbA1.Ray//FX1001///NT22705//FX1001*2/TB1R.FX1001.EMS15 will be crossed with NT22705. FX1001/tbA1.Ray//NT23226///NT23226//FX1001*2/TB1R.FX1001.EMS15 will be crossed with NT23226. Backcrosses will continue to be made using recurrent parents as male pollen donors until male sterility in the F_1 progeny of these crosses is reduced, indicated by consistent seed set in any F_1 spikes that are allowed to self.

Conclusion

An EMS mutagenesis population was successfully created in triticale, a deleterious *TB-R1* mutation was derived, and EMS lines were catalogued for future studies. The *tb-A1-R256** mutant allele was introgressed into triticale from hexaploid wheat based on methods outlined in Hills et al. (2007). This involved using triticale as the female parent in initial F_1 crosses, and subsequently using the recurrent triticale parent as the male pollen donor in backcrosses due to persistent male sterility. A protocol using cold treatments and gibberellic acid solution was developed to aid in the successful germination of F_1 seeds resulting from these crosses. *TB-A1* and *TB-R1* mutant alleles are combined in three different triticale backgrounds to initiate the development of NIL that can be used to test the ability of these alleles to increase tillering capacity in triticale lines. Increased tillering in triticale should increase forage biomass yields,

and potentially grain yields as well. Future studies using developed near isogenic lines will test this hypothesis.

References

Baron, V. S., Juskiw, P. E., & Aljarrah, M. (2015). Triticale as a forage. *Triticale*, 189-212. https://doi.org/10.1007/978-3-319-22551-7_10

Blakeslee, A. F., & Avery, A. G. (1937). Methods of inducing doubling of chromosomes in plants. By treatment with colchicine.

Bruckner, P. L., Berg, J. E., Lamb, P. F., Carr, P., Wichman, D. M., Miller, J. H.,…Chen, X. (2019). Registration of 'Ray' Hard Red Winter Wheat. *Journal of Plant Registrations*, *13*(3), 392-395. https://doi.org/10.3198/jpr2019.03.0012crc

Chaturvedi, G. S., Aggarwal, P. K., Singh, A. K., Joshi, M. G., & Sinha, S. K. (1981). Effect of irrigation on tillering in wheat, triticale and barley in a water-limited environment. *Irrigation Science*, *2*(4). https://doi.org/10.1007/bf00258376

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

Doebley, J., Stec, A., & Gustus, C. (1995). Teosinte Branched1 and the Origin of Maize - Evidence for Epistasis and the Evolution of Dominance. *Genetics*, *141*(1), 333-346. https://doi.org/10.1093/genetics/141.1.333

Doebley, J., Stec, A., & Hubbard, L. (1997). The evolution of apical dominance in maize. *Nature*, *386*(6624), 485-488. https://doi.org/10.1038/386485a0

Hills, M. J., Hall, L. M., Messenger, D. F., Graf, R. J., Beres, B. L., & Eudes, F. (2007). Evaluation of crossability between triticale (X Triticosecale Wittmack) and common wheat, durum wheat and rye. *Environmental biosafety research*, *6*(4), 249-257. https://doi.org/10.1051/ebr:2007046

Krasileva, K. V., Vasquez-Gross, H. A., Howell, T., Bailey, P., Paraiso, F., Clissold, L.,…Dubcovsky, J. (2017). Uncovering hidden variation in polyploid wheat. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(6), E913-E921. https://doi.org/10.1073/pnas.1619268114

Lelley, T. (2006). Triticale: A low-input cereal with untapped potential. *Genetic resources chromosome engineering and crop improvement*, *2*, 395-430.

Lukaszewski, A., & Gustafson, J. (2011). Cytogenetics of triticale. *Plant breeding reviews*, *5*, 41-93.

McGoverin, C. M., Snyders, F., Muller, N., Botes, W., Fox, G., & Manley, M. (2011). A review of triticale uses and the effect of growth environment on grain quality. *Journal of the Science of Food and Agriculture*, *91*(7), 1155-1165. https://doi.org/10.1002/jsfa.4338

McVay, K., Meccage, E. C., Bourgault, M., Carr, P. M., & Boss, D. L. (2018). Potential of annual forages in the Northern Great Plains. Crops & Soils, 0. https://doi.org/10.2134/cs2019.52.0101

Mergoum, M., Singh, P., Pena, R., Lozano-del Río, A., Cooper, K., Salmon, D., & Gómez Macpherson, H. (2009). Triticale: a "new" crop with old challenges. *Cereals*, 267-287. https://doi.org/10.1007/978-0-387-72297-9_9

Whipple, C. J., Kebrom, T. H., Weber, A. L., Yang, F., Hall, D., Meeley, R.,…Jackson, D. P. (2011). *grassy tillers1* promotes apical dominance in maize and responds to shade signals in the grasses. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(33), E506-E512. https://doi.org/10.1073/pnas.1102819108

Wilson, A. (1875). 1875. On wheat and rye hybrids. *Trans, and Proc. Bot. Soc. 12i*, 286-288.