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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, crops are planted at a lower density, and lines with increased tillering potential can improve yields. Drought-tolerant genotypes with higher tillering potential can provide a harvestable crop in poor years while maximizing yields in favorable conditions. Greater tillering potential allows plants to capitalize on timely rainfall. *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 homeolog can enhance tillering potential, boosting yield under favorable conditions. *TB1* variants were analyzed in near-isogenic line populations across 3 years and five Montana environments. Lines with mutations in both *TB1* homeologs 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 a grain yield increase of up to 20% in environments with optimal mid-season rainfall and did not yield significantly lower than the wildtype genotypes in any other environment. Integrating a *TB-B1* nonsense allele into durum wheat breeding programs could be useful to increase productive tillers and yield potential.

Plain Language Summary

This study focuses on wheat, a crop that can handle drought. In areas with more rain or irrigation, planting wheat densely can be beneficial. However, in places with

Abbreviations: EMS, ethyl methanesulfonate; NIL, near-isogenic line; *Rht-1*, *Reduced Height-1*; *TB1*, *Teosinte Branched-1*; TILLING, Targeting Induced Local Lesions in Genomes.

less water and unpredictable weather, wheat needs to be flexible to maintain high yields. The research looks at a gene called *Teosinte Branched-1* (*TB1*), which affects how wheat plants grow extra stems (tillers). By reducing *TB1* function, wheat can grow more tillers, which helps increase grain yield when conditions are good. The study tested different *TB1* variants in durum wheat over 3 years in Montana. Results showed that wheat with mutations in both *TB1* genes had more tillers but sometimes smaller grain yields. However, wheat with a specific *TB1* mutation (*tb-B1-W341**) had up to 20% higher yields in good rainfall conditions without significant yield loss in other environments. Adding this mutation to breeding programs could improve wheat's yield.

1 | 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 (nonirrigated) production, such as the US Northern Great Plains. Durum wheat (*Triticum turgidum* L. subsp. *durum*) is a tetraploid species of wheat primarily grown to produce semolina used in pasta production. The Northern Great Plains is the primary growing region for durum wheat in North America, with production centered in Montana, North Dakota, Saskatchewan, and Alberta, totaling 7.7 million metric tons in 2024, comprising almost one quarter of durum wheat production worldwide (AAFC, 2024; Peterson, 2024; USDA, 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 the absence of the D genome simplifies experimental design. 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 (*Z. 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., 1995, 1997). *TB1* orthologs 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* ortholog 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 (Dixon et al., 2018; Lewis et al., 2008). Dixon et al. (2018) demonstrated that increased *TB1* dosage increases paired spikelets while also decreasing tillers. A 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). 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 homoeologs, 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, less drought-prone 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 when 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 maize 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). C. T. 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 (C. 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 (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* quantitative trait locus (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 stable across different genotypes and environmental conditions. Increasing tillering potential would likely improve grain yield primarily in environments with timely midseason 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.

Core Ideas

- Reducing *Teosinte Branched-1* (*TB1*) function increases tillering.
- *TB1-B* nonsense mutations increase yield potential.
- *TB1* mutations tested in near isogenic over several years.

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 potential that can take advantage of limited water resources in the harsh growing conditions of the Northern Great Plains. 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 locations/years in yield trials and were also planted in variable rate seeding experiments across three locations/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.

2 | MATERIALS AND METHODS

2.1 | Creation of isogenic lines

Two EMS-derived mutant lines were selected from the Kronos TILLING (Targeting Induced Local Lesions in Genomes) database, Kronos994 and Kronos562. These lines contain the nonsense mutant alleles *tb-A1-W339** and *tb-B1-W341**, respectively (Krasileva et al., 2017). The coding sequences of *TB-A1* and *TB-B1* code for a total of 353 and 355 residues, respectively, meaning that mutations in the tested alleles occur toward the end of the coding region. 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). The 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

TABLE 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′

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 (NILs). Seed from these plants was increased in the greenhouse in 30-cm wide pots, with two pots of five seeds planted per each NIL. The 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.

2.2 | 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 polymerase chain reaction (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), 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 s of denaturation at 96°C, 30 s of annealing at 65°C, and 1 min of extension at 72°C. Sequence was analyzed using SeqMan Pro Version 17 (DNASTAR).

Allele-specific qPCR PACE genotyping markers (3CR Bioscience) were later developed to genotype for *tb-A1-W339** and *tb-B1-W341** alleles. Developed primer sequences are shown in Table 1. Each 10 µL reaction contained 1.2 µL of genomic DNA at a concentration of approximately 2 µg μL^{-1}, 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 s 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 s, 57°C of combined annealing and extension for 1 min, and a plate read step at 30°C for 30 s.

2.3 | Field experiments

Experiments examining MT Raska background *TB1* NIL were grown in 11 experiments across four locations and 3 years (Table 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 2022–2024 in two separate yet adjacent experiments with two replicates each. In 2022, one of these experiments was irrigated with handline sprinklers to add approximately 15 cm of irrigation water in 5 cm increments on June 21 and 26 and July 2. In 2023, this location received above-average growing season rainfall (Table 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 July 7. Similar experiments were planted in 2023 and 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. For all planting dates, harvest dates, seeding rates, and row spacing for each trial, see Table 2. for average monthly temperatures and total water received (including irrigation) for each experiment, see Table 3. All trials were amended with nitrogen and phosphorus fertilizer based on available soil nutrients and projected grain yields in each

TABLE 2 Different field management details at different locations and years in which experiments in this study were planted.

Year	Location	Water treatment	Reps (No.)	Rows/plot (No.)	Plot length (m)	Row spacing (cm)	Plot area (m^2)	Seeding rate (seeds m^{-2})	Plant date	Harvest date
2022	Bozeman	Rainfed	2	2	3.0	30	1.9	196	May 6	September 12
2022	Bozeman	Irrigated	2	2	3.0	30	1.9	196	May 6	September 12
2023	Bozeman	Rainfed	4	2	3.0	30	1.9	Multiple[a]	May 5	September 15
2023	Havre	Rainfed	2	3	5.5	30	4.5	233	April 29	August 4
2023	Moccasin	Rainfed	2	5	3.0	30	4.6	224	May 3	August 15
2023	Sidney	Rainfed	2	7	3.0	18	4.6	231	April 18	August 8
2024	Bozeman	Rainfed	2	2	3.0	30	1.9	Multiple[a]	April 24	August 27
2024	Bozeman	Irrigated	2	2	3.0	30	1.9	Multiple[a]	April 16	August 23
2024	Havre	Rainfed	3	3	5.5	30	4.5	233	April 24	August 27
2024	Moccasin	Rainfed	3	5	3.0	30	4.6	224	April 23	August 20
2024	Sidney	Rainfed	3	7	3.0	18	4.6	231	April 15	August 1

[a] In Bozeman seeding rate experiments, plots were planted at a high seeding rate of 391 seeds m^{-2}, a standard seeding rate of 196 seeds m^{-2}, a low seeding rate of 98 seeds m^{-2}, and a very low seeding rate of 42 seeds m^{-2}.

TABLE 3 Monthly precipitation and temperature data for each location and year in which experiments were planted.

				Water received (cm)						Average temperature (°C)					
Year	Location	Water treatment	Last frost (date)[a]	Apr	May	Jun	Jul	Aug	Season total	Apr	May	Jun	Jul	Aug	Season avg.
2022	Bozeman	Rainfed	May 31	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	May 31	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	June 22	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	April 28	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	May 10	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	April 30	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	May 31	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	May 31	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	May 4	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	June 1	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	April 21	0.9	8.8	4.6	1.5	1.3	17.2	12.6	16.2	19.9	25.4	24.4	19.7

Abbreviation: avg., average.

[a] Last frost recorded on the last calendar day on which there was an observed air temperature of <0°C.

[b] No data recorded this month.

region. Trials were treated with appropriate herbicides and pesticides according to field conditions. Seed planted in all 2023 and 2024 trials was treated with CruiserMaxx Vibrance for Cereals (Syngenta).

In 2023 and 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 the standard "high" seeding rate of 391 seeds m^{-2}, a half of the standard "low" rate of 98 seeds m^{-2}, and a slightly less than quarter of the 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 subplot. 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.

In all 11 standard density trials, mature tiller number and productive spike number were recorded from a representative 30-cm section of row. Counts were performed at physiological maturity. Physiological maturity was determined when at least half of the peduncles in a plot had turned brown. The 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 the soil surface to the top of representative primary spikes, not including awns. Before

TABLE 4 Timepoints of tiller counts at different growth stages in Bozeman experiments.

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

harvest, when grain had fully matured (Zadoks stage 92), five spikes from primary tillers were randomly selected from each plot for analysis (Zadoks et al., 1974). Spikes were left to dry fully, the 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 these data, average spikelets per spike, average grain weight per primary spike, average grain number per primary spike, and average grain number per spikelet were 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.), 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 kilogram per hectare 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), 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 2022–2024, tillers were counted within the same section of row at three different timepoints across the growing season to determine if increases in tillering in *TB1* mutant lines occurred earlier or later in plant development. 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 were performed at physiological maturity, as in all other locations. For dates of tiller counts at each growth stage for each year, see Table 4. 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. The heading date and maturity date were recorded for all experiments planted in Bozeman, including all variable seeding rate experiments. The heading date was determined by the Julian date on which 50% of spikes in the plot had fully emerged from the sheath.

2.4 | 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) 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 the 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 was 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.

3 | RESULTS

3.1 | Overall results

A stepwise increase in tiller number was observed on average across all environments, with single *tb-B1-W341** mutant lines and double *tb-A1-W339** and *tb-B1-W341** producing significantly more tillers and productive spikes than the wildtype (Table 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 the wildtype lines (Table 5). Both single-mutant genotypes produced slightly smaller spikes than the 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 the wildtype, although this difference was not statistically significant overall (Table 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 trait (Table 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. For mean values across all environments, see Table 5, and for mean values within each environment, Tables S1–S11.

In 2022, both single-mutant genotypes produced significantly higher grain yield than the 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 S1). 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 S3). 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, and 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 S1).

TABLE 5 Mean agronomic trait values of MT Raska background near-isogenic line (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 (kg ha^{-1})	Mature tillers (tillers m^{-2})	Productive spikes (tillers m^{-2})	Height (cm)	Spikelets/spike (No.)	Single-grain weight (mg)	Yield/spike (g)	Seeds/spikelet (No.)	Grain protein content (g kg^{-1})
TB-A1 allele	*TB-B1* allele									
TB-A1a	*TB-B1a*	4104	547.9	493.0	69.9	14.8	33.6	1.51	2.85	14.0
*tb-A1-W339**	*TB-B1a*	4129	559.7	505.9	67.1	14.9	33.5	1.44	2.71	14.3
TB-A1a	*tb-B1-W341**	4154	580.2	518.8	68.4	14.3	33.6	1.43	2.79	14.1
*tb-A1-W339**	*tb-B1-W341**	4033	603.9	537.1	67.1	14.2	33.3	1.34	2.69	14.4
	LSD$_{0.05}$	**94.7**	**21.6**	**19.3**	**0.51**	**0.16**	0.50	**0.033**	**0.054**	**0.16**
	No. of 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
	G × E	<0.0001	0.150	0.200	0.660	0.729	0.010	0.068	0.142	0.033

Note: The values in bold indicate significance is noted as 0.05.

Abbreviation: LSD, least significant difference.

Plant height differed significantly between genotypes. The *tb-B1-W341** single-mutant lines were 1.5 cm shorter than the 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 the wildtype lines ($p < 0.0001$, independent-sample *t*-test) (Table 5). Although small, this height difference was consistent across all environments (Table S4). All genotypes shared the same heading dates and maturity dates within the 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). 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 S9). There were no significant differences in leaf length or leaf width, although the double-mutant genotypes trended toward having reduced flag leaf length of approximately 1.0 cm, depending on the environment (Tables S10 and S11).

3.2 | 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, Table S12). 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. This overall decrease in tiller number later in the season is typical in drought-prone regions as plants will reallocate nutrients from smaller tillers toward primary tillers during grain fill. The *tb-B1-W341** single-mutant lines generally produced more tillers than the 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 the wildtype lines, although this difference was 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 the wildtype but fewer 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 toward producing fewer tillers than the wildtype across all three timepoints (Figure 1, Table S12).

3.3 | Seeding rate trial results

As noted previously, standard seeding rate experiments (196 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) (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 1–2 days later than lines planted at standard and high seeding rates (data not shown).

4 | DISCUSSION

4.1 | Tillering potential 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 the wildtype, extra tillers wither away later in the growing season without adequate moisture availability. The *tb-B1-W341** single-mutant lines showed increased tillering potential in certain environments. In environments with lower moisture, like the 2022 rainfed Bozeman environment, these lines tillered at similar levels to the wildtype (Figure 1, Table S12). 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

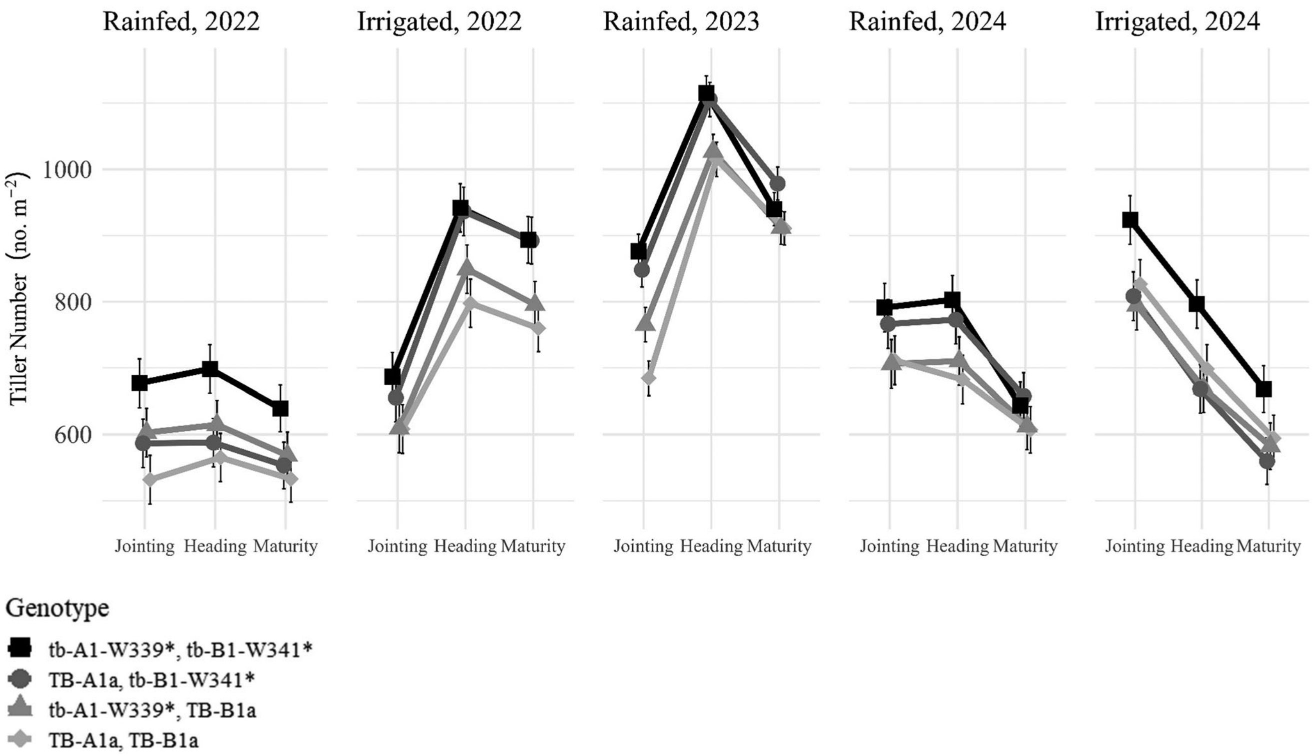

FIGURE 1 Tiller number differences between *Teosinte Branched-1* (*TB1*) genotypes across the growing season in five Bozeman environments. Average tiller numbers of the single-mutant *tb-B1-W341** genotype group relative to other genotype groups highlight the high tillering potential of lines containing this allele.

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 potential allowed for rapid growth and tillering in the month of May, which experienced above average rainfall (Table 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 (Tables 2 and 3). Overall, increased tillering potential 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 inadequate moisture levels later in the season limit yield potential during grain fill, the extent of increased tillering in the single *tb-B1-W341** mutant lines is not sufficient to cause a yield reduction in these conditions relative to the 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 the wildtype (Table S1, Table 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, the 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 keeps 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 growth stages at which the tillering potential of *TB1* mutant lines is most drastically increased. 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 4 days prior to these counts. This irrigation event did not cause an increase in tillers in double *TB1* mutant genotypes relative to the nonirrigated 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 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, Table S12). These differences across environments and years highlight the increased tillering potential of single *tb-B1-W341** mutant lines relative to other genotypes.

The effect of this increased tillering potential on yield can be exacerbated when the 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 suggest that plants with reduced *TB1* function can partially ignore shading cues from neighboring plants and more completely utilize available resources.

4.2 | *TB1* affects height

One finding in conflict with previous studies is the observed stepwise height decrease in *TB1* mutant lines (Table 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). All recurrent parent lines (MT Raska and MT112219) and donor lines (Kronos994 and Kronos562 TILLING mutants) contain identical *Rht-A1a* and *Rht-B1b* alleles, and as such, there are no confounding effects between NILs resulting from the *Rht-1* locus, despite the introgression regions of the tested NIL containing this locus. *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).

4.3 | 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 the 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 handline 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 potential can perform well in lower resource environments.

4.4 | Potential belowground effects

While increases in productive tiller number are observed in environments in which *tb-B1-W341** single-mutant lines yielded significantly higher than the wildtype lines, these increases do not account for the overall magnitude of increase in grain yield. Lacking explanatory differences in the aboveground phenotype point toward differences in the belowground 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.

5 | 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 the 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 potential, these lines can most effectively take advantage of mid-season irrigation or rainfall relative to the wildtype, increasing productivity based on available moisture. If conditions are right, the 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 potential can improve grain yield in durum wheat.

AUTHOR CONTRIBUTIONS

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

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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

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