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Durum wheat *Teosinte Branched1* null mutations increase tillering

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Abstract

Wheat (*Triticum* spp.) yield is increased by either producing and testing variants of yield-limiting genes or crossing to create new allelic combinations. Tiller number and seeds per tiller influence wheat yield, and both are impacted by the *Teosinte Branched1* (*TB1*) gene. We screened 16 hard red spring wheat (*Triticum aestivum* L.) cultivars, 15 hard red winter wheat cultivars and lines, and 10 durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) van Slageren] cultivars and lines commonly grown in Montana and found several previously reported *TB1* missense alleles along with one new silent and a missense *TB1-A1* allele among the 16 hard red spring wheat cultivars but no *TB1* null alleles. Among the 10 durum cultivars, five carried a *TB1-B1-S184G* missense allele and all winter wheat cultivars carried wild-type *TB1* alleles. To determine the impact of *TB1* null alleles, this study examined durum wheat genotypes varying for *TB1* null alleles created by ethyl methanesulfonate (EMS) mutagenesis. The goal was to determine the impact of *TB1* null alleles on tillering, agronomic, and seed traits. The impact of *TB1* null alleles was studied alone and together in field trials. The *TB1* single- and double-null mutants produced up to 15% more tillers and 18% with positive trends toward increased plant biomass. The results of this research demonstrate that the integration of EMS-derived *TB1* null alleles may prove useful in increasing small-grain tillering to optimize plant biomass and yield under different growing conditions.

1 | INTRODUCTION

Durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) van Slageren] is a tetraploid wheat species grown in the United States primarily in North Dakota, Montana, California, and Arizona (National Agricultural Statistics Service [NASS], 2020b). Durum's primary use is in being milled into semolina for pasta production, though other uses exist. Although durum accounted for only 2–4% of total U.S. wheat production area from 2011 to 2020 (NASS, 2020a), it has major economic importance in the areas where it is grown. In 2019, Montana harvested 222,577 ha of durum with a worth of ~US$104 million (NASS, 2019).

Durum breeding program objectives include region-specific trait improvements such as disease resistance in addition to improvements in breeding goals common to all breeding programs such as high seed protein content and semolina yield, gluten strength, firm pasta, and bright yellow semolina and pasta color (Clarke et al., 1998; Royo et al., 2009). Since grain yield is the factor that most influences farmers' profitability, and accordingly their planting choices,

Abbreviations: EMS, ethyl methanesulfonate; PCR, polymerase chain reaction.

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it is important for breeding programs to generate locally adapted, high-yielding durum cultivars.

Environmental and genetic factors both impact total grain yield. Factors, including cold or heat stress, water and nutrient availability, and disease and pest pressure, can negatively impact grain yield, but the genetic component is what determines the yield potential of a given cultivar (Duggan et al., 2000; Destro et al., 2001). Genetically controlled, yield-impacting traits, such as spikelets per head, seed size, and number of productive tillers, are negatively correlated, making selecting for increased yield challenging (Fischer et al., 1977; Cantrell & Haro-Arias, 1986). Considering this, studies focused on increasing yield generally concentrate on just one or a few of these factors. Varying levels of success have been observed with this method from widespread beneficial effects to yield impacts only under certain environmental conditions (Garcia del Moral et al., 2003; Elhani et al., 2007; Moayedi et al., 2009; Destro et al., 2001).

Selection for mutations in genes that both decrease height and increase productive tillers is a proven method to deliver higher yields as seen with the semi-dwarfing alleles, *Rht-B1b* and *Rht-D1b*, which are widely deployed in hexaploid bread wheat (*Triticum aestivum* L.) cultivars. Reducing the height of a plant allows the redirection of energy from stem elongation to seed production (Youseffian et al., 1992; Flintham et al., 1997; Mathews et al., 2006). The *Rht–B1b* semi-dwarfing allele has also been incorporated into durum wheat cultivars. Along with *Rht*-related yield increases from reduced height and increased productive tillers, other genes could also provide a yield increase through similar mechanisms.

An increase in total tiller number, when associated with an increase in productive tiller number, can increase plant yield (Destro et al., 2001; Elhani et al., 2007; Naseer et al., 2016). However, simply increasing total tillers is not sufficient. Late increases in tillering can siphon away limited resources that otherwise would contribute to grain yield, resulting in a plant with few productive tillers and lower grain yield (Elhani et al., 2007; Motzo et al., 2004). Therefore, it is necessary to only increase the number of productive tillers. An allele identified at a chromosome 6B quantitative trait locus was associated with increased early tiller number, productive tiller number, and yield in favorable environments (Naseer et al., 2016). This allele has been identified in several popular Montana-grown hard red spring wheat cultivars, such as 'Vida' (PI 642366) and 'Reeder' (PI 613586) (Naseer et al., 2016), indicating that manipulation of tiller number is a factor already being used in wheat breeding programs. Demonstrating specific genes' effects on tillering is an approach that can be used to modify genetic yield potential.

One such gene that affects tillering is *Teosinte Branched1* (*TB1*). First identified in maize (*Zea mays* L.) as one of the main genes that differentiates modern maize from its progenitor, *TB1* expression in maize decreases the multibranching architecture specific to teosinte (Doebley et al., 1995) and results in the modern single-stalked maize plant having few to no upper branches (Doebley et al., 1997). Homologs of *TB1* have been identified in multiple species: *OsTB1* in rice (*Oryza sativa* L) (Sakamoto & Matsuoka, 2004), *BRC1* in *Arabidopsis thaliana* (L.) Heynh. (Aguilar-Martinez et al., 2007), *SbTB1* in sorghum [*Sorghum bicolor* (L.) Moench] (Kebrom et al., 2006), *PvTB1* in switchgrass (*Panicum virgatum* L.) (Xu et al., 2016), and *TaTB1/TB1* in bread wheat (Lewis et al., 2008; Liu et al., 2017).

As a Class II member of the transcriptional regulator family known as the TCP family (Cubas et al., 1999), *TB1* and its homologs all have conserved function as negative regulators that suppress lateral branching, growth of axillary buds, and internode growth of branches (Aguilar-Martinez et al., 2007; Doebley et al., 1995, 1997; Takeda et al., 2003). However, the effect of *TB1* on plant architecture varies across species. In maize, *TB1* prevents outgrowth of buds at lower nodes and promotes development of ears at upper nodes, while mutant *TB1* plants develop more tillers and male inflorescences (Cubas et al., 1999). Mutant *TB1* alleles in maize were also associated with increased node length and plant height (Hubbard et al., 2002). Maize *TB1* mutants also increased root biomass while keeping the ratio of aboveground to belowground growth similar between mutant and wild-type *TB1* genotypes (Gaudin et al., 2014) along with reduced expression of genes in growth and development regulating hormone pathways (Dong et al., 2019). Overexpression of *Oryza sativa TB1* (*OsTB1*) suppresses bud outgrowth by limiting tiller development to the primordial stage in rice (Sakamoto & Matsuoka, 2004), while overexpression of *Panicum virgatum TB1* (*PvTB1*) in switchgrass inhibited tiller emergence and development along with inducing changes in stem height and diameter and biomass yield (Xu et al., 2016). Repressed *TB1* expression in sorghum was shown to induce axillary bud growth (Kebrom et al., 2006).

Hexaploid wheat containing *TB1* mutant alleles exhibiting reduced in vitro binding to the FT1 (Flowering locus T1) protein were taller than wild-type plants (Dixon et al., 2020). Altered bread wheat inflorescence architecture in the form of paired spikelets was associated with increased D

Core Ideas

- *Teosinte Branched1* null alleles increase wheat tillering.
- Selection for increased tillering can increase wheat biomass.
- *Teosinte Branched1* null impact is influenced by the environment.

TABLE 1 Summary of ethyl methanesulfonate-derived *Teosinte Branched1* (*TB1*) null mutations

Allele	DNA change[a]	Original codon	New codon
TB1-A-W339Stop	1,016	TGG	TAG
TB1-B-W341Stop	1,023	TGG	TGA
TB1-B-Q142Stop	424	CAG	TAG

[a]Nucleotide locations are relative to the coding sequence starting with the start codon.

genome expression of *TB1* (Dixon et al., 2018). Lewis et al. (2008) showed that *TB1* overexpression in wheat results in fewer tillers, reduced axillary bud outgrowth, shorter plants, and increased inflorescence axillary outgrowth. Additionally, overexpression of the tae-miR156 regulator resulted in reduced expression of *Triticum aestivum TB1* (*TaTB1*) and enhanced hexaploid wheat tillering (Liu et al., 2017).

These studies provide the basis for our research. Overexpression of *TB1* is associated with decreased tillering (Xu et al., 2016; Sakamoto & Matsuoka, 2004), while wild-type *TB1* (and orthologs) plants exhibit apical dominance with few main branches (Aguilar-Martinez et al., 2007; Doebley et al., 1995, 1997; Takeda et al., 2003) and mutant *TB1* plants have increased tillers (Cubas et al., 1999; Xu et al., 2016; Kebrom et al., 2006; Liu et al., 2017) and increased height (Dixon et al., 2020). We hypothesized that tiller number can be increased leading to increased total biomass and grain yield in durum and other related wheat species by suppressing *TB1* expression by selecting for *TB1* null alleles.

We first screened commonly grown Montana winter, spring, and durum wheat genotypes to determine the level of natural *TB1* allelic variation and found no naturally occurring *TB1* null alleles. An ethyl methanesulfonate (EMS) mutagenized durum wheat population was then screened for *TB1* stop codon mutations. Three lines containing *TB1* stop mutations were chosen for crossing, one *TB1-A* and two *TB1-B* null alleles, to create segregating populations. Field trials were conducted on the populations created from those crosses to evaluate phenotypic differences in tiller and other plant morphological and agronomic traits.

2 | MATERIALS AND METHODS

2.1 | Plant material

2.1.1 | Variety screen

Fifteen winter wheat cultivars, 15 spring wheat cultivars and experimental lines, and 10 durum wheat cultivars and experimental lines (Supplemental Table S1) along with controls of 'Chinese Spring' wheat and one representative line of each of the four genotypes of interest ('Kronos' AABB, aabb, AAbb, and aaBB) were planted in the greenhouse then DNA extracted at the two-leaf stage. Each line was genotyped with primers designed to capture the whole *TB1* gene (see Supplemental Table S2 for primer sequences and product size). Polymerase chain reaction (PCR) conditions were as follows: initialization 96 ˚C for 5 min, then 40 cycles of 96 ˚C for 30 s, 30 s at the annealing temperature shown in Supplemental Table S2, 1 min at 72 ˚C, and a 7-min final extension at 72 ˚C. PCR products were Sanger sequenced by GENEWIZ (GENEWIZ, Inc.), then the sequence was analyzed for differing alleles using SeqMan Pro software v15.1 (DNASTAR) against the reference genes from Liu et al. (2017).

2.1.2 | EMS-derived *TB1* null alleles

The wheat TILLING (Targeting Induced Local Lesions IN Genomes) population database (Tsai et al., 2011; Krasileva et al., 2017) was screened using online tools for *TB1* null mutations in the durum wheat 'Kronos' (PI 576168) and three lines containing *TB1* stop mutations were chosen for this project: *TB1-A1-W339**, *TB1-B1-W341**, and *TB1-B1-Q142** (Table 1). The M_4 seed of those lines was obtained and M_4 plants grown in fall 2018. The F_1 crosses between the *TB1-A1-W339** line and both *TB1-B1* mutant lines were made and the resulting F_1 seeds were grown and allowed to self-pollinate. DNA was extracted from the F_2 plants and PCR was performed using *TB1* gene-specific primers (Table 2). Conditions for PCR were 40 cycles of 30 s denaturation at 96 ˚C, 30 s annealing at 65 ˚C, and 60 s extension at 72 ˚C for both primer sets. The PCR products were Sanger sequenced (GENEWIZ, Inc.) and analyzed using SeqMan Pro v15.1 (DNASTAR) against the reference genes from Liu et al. (2017). Only *TB1* homozygous mutant and wild-type F_2 plants were advanced.

In this manner, four genotypes of interest for each cross were created. These were TB1-A1, TB1-B1 (AABB), TB1-A1, TB1-B1-Q142* (AAbb), TB1-A1-W339*, TB1-B1 (aaBB) and TB1-A1-W339*, and TB1-B1-Q142* (aabb) for the TB1-A1-W339* × TB1-B1-Q142* cross. For the other cross, the genotypes were TB1-A1, TB1-B1 (AABB), TB1-A1, TB1-B1-W341* (AAbb), TB1-A1-W339*, TB1-B1 (aaBB), and TB1-A1-W339*, TB1-B1-W341* (aabb) for the TB1-A1-W339* × TB1-B1-W341* cross. The F_2 homozygotes were grown and allowed to self-pollinate to produce seed of F_2–derived F_3 lines for planting in the field during spring 2019. The number of F_2–derived F_3 lines varied from eight to 18 in each of the four genotype classes for each cross.

2.1.3 | Field experiments

The F_2–derived F_3 lines (102 entries) were grown in two replications of a randomized complete block design at the

TABLE 2 Amplification and sequencing primers used to detect *Teosinte Branched1* (*TB1*) mutations

Mutation detected	Primer sequence (5′→3′)[a]	Primer type	Amplicon size
			bp
*TB1-A1-W339**	GATCGACAACCAGCCGCCA	Forward	862
	TTTCCCAACTCGCAGCCC	Reverse	
	GACAAGGAGTCGAGGACG	Sequencing	
*TB1-B1-W341**	GATCGACAACCAGTCGCCG	Forward	859
	TTTCCCAACTCCCAGCCC	Reverse	
	GACAAGGAGTCGAGGACG	Sequencing	
*TB1-B1-Q142**	GATCGACAACCAGTCGCCG	Forward/	859
		Sequencing	
	TTTCCCAACTCCCAGCCC	Reverse	

[a]Primers developed from sequence alignment of accession numbers TraesCS4A02G271300 and TraesCS4B02G042700.

Arthur H. Post Agronomy Farm near Bozeman, MT (45.67° N, 111.15° W, 1,455 m asl, soil type is Amsterdam silt loam [fine-silty, mixed, superactive, frigid Typic Haplustolls]). The plants were grown in 1.5-m rows with 30-cm spacing between each row. Within a row, seeds were sown 15 cm apart with 13 plants per row. Seeds were hand planted to a depth of 2.5 cm on 9 May 2019. After testing soil, 185 kg ha^{-1} urea (46-0-0) was added. From 1 May to 31 August, the research station received 18.62 cm of precipitation. The highest recorded air temperature occurred on 23 July at 32.3 °C, the lowest recorded air temperature was −4.18 °C on 1 May. Five centimeters irrigation water was applied using hand line sprinklers on 1 July. A 1.1 L ha^{-1} application of Huskie (Pyrasulfotole 3.3%, Bromoxynil Octanoate 13.4%, Bromoxynil Heptanoate 12.9%; Bayer CropScience) was applied for broadleaf weed control on 10 June, and throughout the growing season weeds were rogued out by hand.

The F_2–derived F_4 seed harvested from the 2019 trial was used for similar field trials in 2020 at the Arthur H. Post Agronomy Farm and at the Central Agricultural Research Center near Moccasin, MT (47.06° N, 109.96° W, 1,297 m asl, soil type Danvers [fine, smectitic, frigid Vertic Argiustolls]–Judith [fine-loamy, carbonatic, frigid Typic Calciustolls] clay loam). Experimental design at both locations was a randomized complete block design with two replications with 93 entries per replication. Plots were 1.5-m rows with 30-cm spacing between rows. Within rows, seeds were sown 15 cm apart with eight plants per row. Plots were hand planted to a depth of 2.5 cm on 25 April 2020 at the Bozeman location and on 11 May 2020 at the Moccasin location.

The Moccasin location was fertilized with 67.3 kg ha^{-1} Environmentally Smart Nitrogen slow-release nitrogen fertilizer (44-0-0, Nutrien) and in Bozeman with 403.5 kg ha^{-1} urea (46-0-0). The Bozeman location received 5 cm irrigation water on 2 July, while the Moccasin location received no added irrigation. From 1 May to 31 August, the Bozeman location received 14.3 cm of precipitation. The highest recorded air temperatures occurred 17 and 18 August at 34.4 °C, the lowest recorded air temperature was −0.6 °C on 8 and 11 May. The Moccasin location received 19.5 cm of precipitation from 1 May to 31 August. The highest recorded air temperature was on 18 August at 36.1 °C, the lowest recorded air temperature was −1.1 °C on 12 May. Herbicides applied to the Bozeman location were 0.06 L ha^{-1} of Affinity Broadspec (Thifensulfuron-methyl 25%, Tribernuron-methyl 25%; FMC), 0.58 L ha^{-1} a MCPA Ester 4 (2-ethylhexyl ester of 2-methyl-4-chlorophenoxyacetic acid 69.7%; Albaugh, LLC), 1.2 L ha^{-1} Discover NG (Clodinafop-propargyl 6.4%; Syngenta Crop Protection, LLC), and 0.29 L ha^{-1} Propi-Star EC (Propiconazole 41.8%; Albaugh, LLC) applied on 1 June. In Moccasin, Vendetta (Octanoic acid ester of bromoxynil 31.7%, 2-Ethylhexyl ester of 2-methyl-chlorophenoxyacetic acid 34%; Wilbur-Ellis Agribusiness) was applied on 27 May for weed control.

2.1.4 | Plant measurements

Plants were measured near physiological maturity after growth had ceased. Plant height was determined by measuring from the soil surface to the terminal spikelet of the tallest tiller. Tiller number was counted at the crown of the plant regardless of whether the tiller produced a head. Spikelets per head was counted on the tallest tiller only. Flag leaf length was measured from stem to tip, and width was measured at the widest point of the leaf. Number of productive heads included only heads that formed seed. Biomass was measured at harvest by weighing the whole plant after cutting at the base. Plants were threshed to obtain grain yield. Straw weight was the difference between biomass and grain yield. Weights were adjusted for moisture content. Seed protein and moisture were measured using a Foss Infratec 1241 Machine (Foss Analytics; AACCI Method 39-11.01). Individual seed weight was measured with a Single-Kernel Characterization System 4100 (Perten, AACCI Method 55-31.01).

TABLE 3 List of variant *Teosinte Branched1* (*TB1*) alleles present in durum and bread wheat cultivars commonly grown in Montana

Allele	DNA change[a]	Original codon	New codon	Provean score[b]	Cultivar
TB1-A1-A104A	312	GCT	GCC	NA	'Alum', 'WB-Gunnison'
TB1-A1-A314P	940	GCT	CCT	−0.952	'SY-Valda'
TB1-B1-S184G	550	AGC	GGC	−2.071	'Riveland', 'Joppa', 'Tioga', 'Precision', 'Mountrail'
TB1-B1-A271V[c]	812	GCA	GTA	−0.061	'McNeal', 'Egan'
TB1-B1-L340L[c]	912	TTG	TTA	NA	'McNeal', 'Egan'
TB1-B1-G66G[c]	198	GAG	GAA	NA	'McNeal', 'Egan'
TB1-B1-S103R[c]	309	AGC	AGG	−0.291	'McNeal', 'Egan'
TB1-B1-K221N[c]	663	AAA	AAT	−1.112	'McNeal', 'Egan'
TB1-D1-D110Y	328	GAC	TAC	−7.939	'Duclair', 'Vida', 'Choteau', 'NS-Presser'

[a]Nucleotide locations are relative to the coding sequence starting with the start codon.
[b]Provean (protein variation effect analyzer) score used to predict effect: A score of −2.5 for single amino acid substitutions is predicted to be deleterious 80% of the time, with higher scores having a deleterious effect less often and lower scores more often.
[c]These five mutations make up the TB-B1b allele reported by Dixon et al. (2018).

Field data was measured by using five consecutive plants from the middle of each row. The five plants were tagged prior to measurements to ensure consistency then harvested together.

2.2 | Statistical analysis

The genotype classes were first compared with ANOVA for each separate environment and then combined over environments, where each cross was analyzed separately. The model for each environment included block genotype and line within genotype class using PROC MIXED in SAS v9.4. The lines within genotype class were considered random and other factors as fixed. Similarly, the model combined over environments included environment, block within environment genotype class, environment × genotype class, lines within genotype class, and environment × lines within genotype class. Individual lines within genotype class and their interaction with location were considered random and other factors were fixed. Since error variances were not homogeneous across environments, we used the group equals environment option on the random statement to accommodate heterogeneous error variances. We compared group means for each environment and combined over environments using *t* tests but only following a significant *F* test ($P < .05$) to protect against Type I errors.

3 | RESULTS

3.1 | *TB1* allele screen

Forty wheat cultivars and lines commonly grown in MT were genotyped for mutations in *TB1* (Supplemental Table S1). One *TB1-A1* silent mutation was present in the hard red spring wheat 'Alum' and 'WB Gunnison', and one *TB1-A1* missense mutation was found in 'SY Valda' (Table 3). The hard red spring wheat cultivars 'McNeal' and 'Egan' carried the *TB-B1b* allele previously reported by Dixon et al. (2018) consisting of two silent mutations and three missense mutations with predicted neutral effects (Table 3). No *TB1* allelic variation was identified among the winter wheat varieties. Five durum cultivars carried a *TB1-B1* missense allele, and four spring wheat cultivars had a predicted deleterious missense mutation in the D genome (*TB1-D1-D110Y*) identified previously by Dixon et al. (2018) (Table 3). No *TB1* null alleles were identified in any of the cultivars or lines screened.

3.2 | Field trials

To test the impact of *TB1* null mutations on durum plant growth under field conditions, we identified *TB1-A1* and *TB1-B1* null mutations using the Kronos tilling population and intercrossed them. The $F_{2:3}$ homozygous lines were grown in 2019 at one location, and $F_{2:4}$ lines derived from the 2019 trial were grown at two locations in 2020.

Location effects were detected for all traits, but the location × genotype class interaction was not significant ($P < .05$) with the lone exception being for tillers per plant in the *Tb1-A1-W339*/Tb1-B1a* × *Tb1-A1a/Tb1-B1-Q142** cross. The double-mutant class had more total tillers and more productive tillers ($P < .05$) than the wild-type class in both crosses (Tables 4 and 5). Significant differences were detected in the 2019 environment ($P < .01$) but not in either 2020 environment even though the rank order of class means was the same. The double-mutant class had shorter flag leaves than the double-wild-type class in both crosses when

TABLE 4 Comparison of agronomic trait measurements taken on each of the four genotypes produced in the Tb1-A1-W339*/Tb1-B1a × Tb1-A1a/Tb1-B1-Q142* cross from field trials combined across three Montana environments

Tb1 genotype	N[a]	Plant height	Tillers	Spikelets	Productive heads	Flag leaf length	Flag leaf width
		cm	No. plant^{-1}	No. head^{-1}	No. plant^{-1}	cm	
TB1-A1, *TB1-B1*	11	62.2 ± 0.98	14.5 ± 0.54b	14.9 ± 0.12	11.4 ± 0.54b	18.3 ± 0.25a	1.45 ± 0.02
TB1-A1, *TB1-B1-Q142**	10	62.5 ± 0.82	15.2 ± 0.58b	14.6 ± 0.13	12.2 ± 0.58b	17.9 ± 0.25a	1.43 ± 0.02
*TB1-A1-W339**, *TB1-B1*	11	64.7 ± 0.93	15.1 ± 0.53b	14.8 ± 0.10	12.2 ± 0.55b	17.3 ± 0.24b	1.44 ± 0.01
*TB1-A1-W339**, *TB1-B1-Q142**	10	65.1 ± 1.01	16.7 ± 0.76a	14.4 ± 0.15	13.4 ± 0.71a	16.9 ± 0.25b	1.41 ± 0.02
Prob > *F*	–	0.22	0.026	0.13	0.017	<0.001	0.68

Note. 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.

[a]Denotes the number of lines in each genotype class.

averaged over environments. That difference was observed in all three environments for the *Tb1-A1-W339*/Tb1-B1a* × *Tb1-A1a/Tb1-B1-Q142** cross (Supplemental Table S3) but only in the 2019 Bozeman environment for the *Tb1-A1-W339*/Tb1-B1a* × *Tb1-A1a/Tb1-B1-W341** cross (Supplemental Table S4). The double-mutant class had fewer spikelets ($P < .01$) than double-wild-type in the Bozeman 2020 environment for the *Tb1-A1-W339*/Tb1-B1a* × *Tb1-A1a/Tb1-B1-Q142** cross and for the *Tb1-A1-W339*/Tb1-B1a* × *Tb1-A1a/Tb1-B1-W341** cross in the Bozeman 2019 environment (Supplemental Tables S3 and S4).

4 | DISCUSSION

The TB1 allele survey conducted on spring, winter, and durum wheat cultivars and lines commonly grown in Montana identified several alleles previously reported to be present in hexaploid wheat cultivars (Table 3). While that variation may be associated with altered *TB1* function, no *TB1* null alleles that could be assumed to have no *TB1* function were identified, and we therefore used *TB1* null alleles since our goal was to determine how *TB1-A* and *TB1-B* null mutations alone and in combination impact tillering and agronomic traits in durum wheat under field conditions. Two null mutations in *TB1-B1*, *TB1-B1-Q142**, and *TB1-B1-W341** and one in *TB1-A* and *TB1-A1-W339** were identified from an EMS population. The two *TB1-B* null mutant lines were each crossed to the *TB1-A* null mutant line to create segregating populations with none, one, or two *TB1* null mutations. Field tests showed the double-mutant class produced more total and more productive tillers per plant than the double-wild-type class in both crosses when averaged over environments (Tables 4 and 6). The single null genotype class means were essentially equal and intermediate between the wild type and double-mutant class means for both total tiller number and productive heads per plant, indicating the A and B genome null mutations may be additive. The differences were much more pronounced in the Bozeman 2019 environment than either of the two 2020 environments (Supplemental Tables S3 and S4). The reason is unknown, but the 2019 Bozeman environment did show more total and productive tillers across all genotype classes than either of the 2020 environments. These results are consistent with our hypothesis that genotypes with *TB1* null alleles could produce more tillers than their wild-type counterparts. Previous studies where *TB1* expression was associated with changes in tiller number in bread wheat were not conducted under field conditions (Lewis et al., 2008; Dixon et al., 2018).

The only other trait to show significant differences among genotype classes when averaged over environments was for flag leaf length where the double-mutant class had shorter flag leaves than the double-wild-type class for both crosses (Tables 4 and 5). Other studies varying *TB1* dosage or allelic variation did not measure leaf morphology traits. Dixon et al. (2020) found increased *TB1* dosage produced plants that had shorter internodes and were shorter than wild type and reciprocally that lines possessing the *TB-B1b* allele, which has multiple point mutations, were taller than those with *TB-B1a*. Our results also showed a trend toward increased plant height with increasing number of null alleles, but that differences did not reach statistical significance (Tables 5 and 7).

Dixon et al (2018) found increased *TB1* dosage altered inflorescence architecture to give paired spikelets. This would ultimately give more spikelets on a single inflorescence. Given those results, null mutations might reduce spikelets per head. We found the double-mutant class did have fewer spikelets per head than the double-wild-type class in one of the three environments for each of our crosses (Supplemental Tables S1 and S2) though that environment was different for each cross.

Results show *TB1* null mutations have the potential to increase tiller number in durum wheat, but the expression of the tiller response can vary depending on the environment. The increase in tiller number did not translate to increased

TABLE 5 Comparison of agronomic trait measurements taken on each of the four genotypes produced in the Tb1-A1-W339*/Tb1-B1a × Tb1-A1a/Tb1-B1-W341* cross from field trials combined across three Montana environments

Tb1 genotype	N[a]	Plant height	Tillers	Spikelets	Productive heads	Flag leaf length	Flag leaf width
		cm	No. plant^{-1}	No. head^{-1}	No. plant^{-1}	——————cm——————	
TB1-A1, *TB1-B1*	16	69.7 ± 0.83	14.9 ± 0.42b	15.0 ± 0.11	12.0 ± 0.42b	20.2 ± 0.22a	1.48 ± 0.01
TB1-A1, *TB1-B1-W341**	18	69.8 ± 0.78	15.9 ± 0.48ab	14.9 ± 0.88	12.6 ± 0.48b	20.2 ± 0.19a	1.48 ± 0.01
*TB1-A1-W339**, *TB1-B1*	8	69.3 ± 1.17	15.8 ± 0.66ab	14.6 ± 0.19	12.5 ± 0.68b	19.4 ± 0.32b	1.53 ± 0.02
*TB1-A1-W339**, *TB1-B1-W341**	9	68.4 ± 1.16	16.7 ± 0.74a	14.5 ± 0.13	13.4 ± 0.67a	19.4 ± 0.31b	1.47 ± 0.02
Prob > *F*	–	0.51	0.04	0.14	0.008	0.02	0.23

Note. 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.

[a]Denotes the number of lines in each genotype class.

TABLE 6 Comparison of seed trait measurements taken on each of the four genotypes produced in the Tb1-A1-W339*/Tb1-B1a × Tb1-A1a/Tb1-B1-Q142* cross from field trials combined across three Montana environments

TB1 Genotype	N[a]	Straw yield	Grain yield	Protein	Seed Weight	Biomass	Harvest Index
		———g plant^{-1}———		%	mg seed^{-1}	g plant^{-1}	
TB1-A1, *TB1-B1*	11	20.4 ± 1.11	19.9 ± 1.39	14.3 ± 0.11	42.5 ± 0.88	40.4	0.48 ± 0.02
TB1-A1, *TB1-B1-Q142**	10	21.3 ± 0.96	20.7 ± 1.25	14.4 ± 0.16	44.3 ± 0.77	41.9	0.49 ± 0.02
*TB1-A1-W339**, *TB1-B1*	11	20.8 ± 0.95	20.5 ± 1.30	14.4 ± 0.18	42.7 ± 0.68	41.4	0.49 ± 0.02
*TB1-A1-W339**, *TB1-B1-Q142**	10	22.6 ± 1.45	20.5 ± 1.29	14.2 ± 0.28	41.9 ± 1.24	43.0	0.48 ± 0.02
Prob > *F*	–	0.77	0.95	0.83	0.33	0.87	0.88

Note. Values represent the average for each genotype ± the standard error.

[a]Denotes the number of lines in each genotype class.

TABLE 7 Comparison of seed trait measurements taken on each of the four genotypes produced in the Tb1-A1-W339*/Tb1-B1a × Tb1-A1a/Tb1-B1-W341* cross from field trials combined across three Montana environments

Tb1 genotype	N[a]	Straw yield	Yield	Protein	Seed weight	Biomass	Harvest index
		———g plant^{-1}———		%	mg seed^{-1}	g plant^{-1}	
TB1-A1, *TB1-B1*	16	27.2 ± 0.79	23.8 ± 0.94	14.7 ± 0.14	49.4 ± 0.98	51.0	0.46 ± 0.01
TB1-A1, *TB1-B1- W341**	18	29.8 ± 0.96	23.5 ± 0.96	14.8 ± 0.18	50.0 ± 0.86	53.2	0.44 ± 0.01
*TB1-A1-W339**, *TB1-B1*	8	27.3 ± 1.08	23.8 ± 1.11	15.2 ± 0.16	49.5 ± 1.24	51.2	0.46 ± 0.02
*TB1-A1-W339**, *TB1-B1-W341**	9	31.4 ± 2.28	21.9 ± 1.25	15.2 ± 0.23	51.1 ± 0.89	53.2	0.42 ± 0.02
Prob > *F*	–	0.220	0.18	0.15	0.46	0.63	0.12

Note. Values represent the average for each genotype ± the standard error.

[a]Denotes the number of lines in each genotype class.

grain yield, but the *TB1* null mutations did not negatively affect any other agronomic trait. Increased grain yield might be achieved by combining the *TB1* null mutations with alleles of genes known to increase seeds per head and seed weight. Genetic materials used in field trials were derived from EMS-derived parents. Background mutations in the parent lines should have segregated equally across the genotype classes. But the background mutations probably contributed to less vigorous plants. The true impact of the *TB1* mutations on tiller number and other agronomic traits will need to be confirmed in near-isogenic lines under densely seeded conditions across multiple environments.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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