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Novel alleles of MFT‐A and MFT‐B1 appear to impact wheat preharvest sprouting in Triticum aestivum and Triticum turgidum ssp. durum

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NOTE

Novel alleles of *MFT-A* and *MFT-B1* appear to impact wheat preharvest sprouting in *Triticum aestivum* and *Triticum turgidum* ssp. durum

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Abstract

Background and Objectives: Preharvest sprouting (PHS) is the premature germination of seeds, which is often caused by late-season rains after seeds reach physiological maturity. PHS negatively impacts grain yield and end-use quality. Previous studies in spring bread wheat (*Triticum aestivum*) and durum wheat (*Triticum turgidum*) have identified that some mutations in the mother of FT and TFL1 gene (*MFT*) coding sequence decrease seed dormancy and increase wheat PHS.

Findings: Here, we report two novel alleles for the *MFT*-A and two novel alleles for the *MFT-B1* homologs in spring bread wheat and durum wheat.

Conclusions: A haplotype analysis suggests that *TaMFT-3A1b* (OQ729929), *TaMFT-3B1b* (OQ729932) and *TdMFT-3B1b* (OQ729937) increase PHS susceptibility. It is expected that functional copies of *MFT* promote seed dormancy. Variant analysis of the novel *MFT-A* and *MFT-B1* alleles in both spring and durum wheat suggest impairment of protein function, therefore a negative impact on seed dormancy.

Significance and Novelty: Previously unassessed durum wheat varieties were examined for PHS susceptibility. The information in this study can serve as a resource for spring and durum wheat breeders to make selections for alleles of *MFT* that impact susceptibility to PHS.

KEYWORDS
durum, mother of FT and TFL1 (MFT), preharvest sprouting, PHS, wheat

1 | INTRODUCTION

Worldwide production of wheat (*Triticum aestivum* & *Triticum turgidum*) in 2021 resulted in approximately 771 million metric tons of grain, making wheat the fourth largest global crop (FAOSTAT, 2023). Spring and winter bread wheat (*T. aestivum*) are utilized in many processed food products, including breads, noodles, cookies, and pastries, while durum wheat (*T. turgidum*) is utilized almost exclusively to make semolina for pasta. Spring bread wheat is hexaploid, containing the A, B, and D subgenomes, while durum wheat is tetraploid, a descendant of the progenitor of spring bread wheat, and contains only the A and B subgenomes. The global

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wheat market is dependent on large quantities of high-quality wheat grain. However the phenomenon known as preharvest sprouting (PHS) can create a significant negative impact on wheat yield and grain quality (Bettge, 2018; Kweon et al., 2023). PHS is caused by late-season rains that occur after physiological maturity and before harvest, resulting in the sprouting of the grain in the wheat head before harvest (Vetch et al., 2019b).

While late-season rains cannot be controlled, there is a genetic component to PHS genes that impacts seed dormancy. Greenhouse studies on the QTL which contains the mother of FT and TFL1 gene (*MFT*), on the A genome, have attributed as much as 58% of the PHS variation in hexaploid wheat to genetic control (Liu et al., 2008). Allelic variation in the A and B2 homologous copies of *MFT* significantly impacts PHS in hexaploid winter bread wheat, but each *MFT* copy only accounts for 18% of the variation for PHS in field studies (Vetch et al., 2019a, 2022). This difference in the proportion of variation contributed by genetics is reflective of the large environmental impact on PHS. Field studies deal with rainfall directly interacting with the developing wheat head during grain fill, while greenhouse studies do not have this issue. *MFT* encodes a phosphatidyl ethanolamine binding protein (PEBP) with seed-specific expression (Chardon & Damerval, 2005). *MFT* impacts seed germination through interactions in the abscisic acid and gibberellin signaling pathways (Xi et al., 2010). Studies of *MFT-A* allelic variants have demonstrated that alleles with higher expression suppress germination (Nakamura et al., 2011). This established the hypothesis that *MFT* functional copies promote seed dormancy, while nonfunctional or less-functional *MFT-containing* genotypes are more susceptible to PHS germination.

In this study, a novel allele of *MFT-A* and *MFT-B1* is reported in spring bread wheat, as well as a novel allele of *MFT-A* and *MFT-B1* in durum wheat. Additionally, PHS scores for Montana-adapted spring bread wheat lines are reported, as well as PHS scores and *MFT* alleles for a global array of durum wheat lines.

2 | METHODS

2.1 | Plant materials and field experiment

Spring bread wheat utilized in this experiment consisted of a subset of 13 elite Montana-adapted cultivars and 41 advanced breeding lines (Supporting Information S1: Table S2), planted at the Montana State University Post Agronomy Farm in Bozeman, Montana, as part of an advanced yield trial in the summers of 2016 and 2017. Lines were planted in three-row plots that were 4 m long with row spacing of 30 cm in an 8×8 partially balanced, incomplete block, triple lattice design. There was 14.7 cm of rain in the 2016 growing season and 13.5 cm of rain in the 2017 growing season. Less than half an inch of rain fell in the 2 weeks preceding harvest each season. There were no visible signs of PHS for any of the wheat grown at the Post Agronomy Farm in either experimental year. No supplemental irrigation was applied in either year. Planting in the 2016 season occurred on April 20th and heads were collected for PHS assessment at physiological maturity immediately before harvesting on August 17th. Planting in the 2017 season occurred on May 6th and heads were collected at physiological maturity immediately before harvesting on August 21st. Nine of the 13 elite Montana-adapted cultivars and 12 of the advanced breeding lines were selected for PHS assessment.

Durum wheat (*T. turgidum*) utilized in this experiment consisted of 15 elite-adapted cultivars from Montana and bordering areas of North Dakota and Canada, two advanced Montana breeding lines, and 15 elite cultivars from around the world (Supporting Information S1: Table S3). The 32 lines were planted at the Post Agronomy Farm in Bozeman, Montana, on May 18, 2022. For each line, 10 seeds were planted in single plant rows, spaced 15 cm apart, with 30 cm between rows, in a randomized complete block design with three reps. There was 11.7 cm of rain from the time of planting until heads were collected for PHS assessment at physiological maturity during the first week of September and a week after physiological maturity during the second week of September. Less than half an inch of rain occurred in the 2 weeks preceding harvest in 2022. There were no visible signs of PHS for any of the wheat grown at the Post Agronomy Farm in the 2022 experimental year. Supplemental irrigation was applied in May and June with 5 cm applied.

2.2 | Genotyping

Three centimeters samples of leaf tissue were collected and bulked from three plants of each cultivar 2 weeks after germination in each field season. DNA extracts of bulked leaf tissue were created utilizing the protocol adapted from Riede and Anderson (1996). Homologs A and B1 of *MFT* were amplified utilizing the polymerase chain reaction (PCR) and the amplicons were sequenced utilizing standard Sanger Sequencing by Azenta Genewiz, South Plainfield, NJ. Sequences from Genewiz were analyzed for variants utilizing the SeqMan Pro tool, a part of the Lasergene DNAStar software suite (DNAStar).

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There are four exons in *MFT*, which together encode a 175 amino acid protein. The first three exons and two introns span 725 base pairs, with the intron between exons 3 and 4 spanning 2138 base pairs, followed by a final exon of 229 base pairs (Vetch et al., 2019b). Given the large size of the third intron, copies of *MFT* were amplified in pieces, targeting the exon regions of the gene. Due to the sequence similarity between *MFT* orthologues in spring bread and durum wheat, the same PCR primers (Supporting Information S1: Table S1) and assay protocol were utilized for both species.

Screening of the *MFT-A* gene sequence was performed utilizing two sets of primers that covered all exons in the gene. A 3531-bp fragment of *MFT-3A* was amplified using forward primer 5′ GTAGCGGGTGAAATCTGCAT 3′, previously published by Nakamura et al. (2011), and reverse primer 5′ CTTGCGAAGAAAAAGCG 3′ (Supporting Information S1: Table S1), which amplified the entire gene as well as a portion of the promoter region where a previously important SNP had been identified (Nakamura et al., 2011). The amplicon was sequenced using the forward primer to obtain the sequence of the *MFT-A* promoter region. The PCR amplification reaction was as follows: one cycle of 96°C for 5 min, 40 cycles of 96°C for 30 s, 61°C for 30 s and 72°C for 240 s, and one cycle of 72°C for 7 min with a final hold at 4°C. The product of the above reaction was diluted 1:100 in deionized H_20 and utilized as the template DNA in the subsequent internal amplification reactions. Targeting the first three exons, a 730-bp internal fragment of the whole gene amplicon was amplified using forward primer 5′ TTTGTACAGGTCGTCGTTGG 3′ and reverse primer 5′ GTGAGAGACACGCAAGAACG 3′ (Supporting Information S1: Table S1), previously published by Liu et al. (2013). The amplicon was sequenced using both the forward and reverse primers to ensure full coverage of the first three exons. The PCR amplification reaction was as follows: one cycle of 96°C for 5 min, 40 cycles of 96°C for 30 s, 59°C for 30 s and 72°C for 90 s, and one cycle of 72°C for 7 min with a final hold at 4°C. Targeting the fourth exon, a 708-bp internal fragment of the whole gene amplicon was amplified using forward primer 5′ TGTAGTCCGCATTGAAATA 3′ and reverse primer 5′ CTTGCGAAGAAAAAGCG 3′ (Supporting Information S1: Table S1). The amplicon was sequenced using the reverse primer. The PCR amplification reaction was as follows: one cycle of 96°C for 5 min, 40 cycles of 96°C for 30 s, 56°C for 30 s and 72°C for 90 s, and one cycle of 72°C for 7 min with a final hold at 4°C.

Screening of the *MFT-B1* gene was performed utilizing two sets of primers that covered all exons in the gene. A 3468-bp fragment of *MFT-3B1* was amplified using forward primer 5′ CGAGCTGTACATGCATGAT 3′ and reverse primer 5′ CTCAGCCTTGTGGTAATAAG 3′ (Supporting Information S1: Table S1), which amplified the entire gene. The PCR amplification reaction was as follows: one cycle of 96°C for 5 min, 40 cycles of 96°C for 30 s, 59°C for 30 s and 72°C for 240 s, and one cycle of 72°C for 7 min with a final hold at 4°C. The product of the above reaction was diluted 1:100 with deionized H_20 and utilized as template DNA in the subsequent internal amplification reactions. Targeting the first three exons, a 688-bp internal fragment of the whole gene amplicon was amplified using forward primer 5′ CATATATACACGCAGGCGT 3′ and reverse primer 5′ CTTATTGGGAGAGTGCAATG 3′ (Supporting Information S1: Table S1). The amplicon was sequenced using both the forward and reverse primers to ensure full coverage of the first three exons. The PCR amplification reaction was as follows: one cycle of 96°C for 5 min, 40 cycles of 96°C for 30 s, 61°C for 30 s and 72°C for 80 s, and one cycle of 72°C for 7 min with a final hold at 4°C. Targeting the fourth exon, a 639-bp internal fragment of the whole gene amplicon was amplified using forward primer 5′ CTTTGTAGGAGTATGTGGTTC 3′ and reverse primer 5′ TGGGTTCGAGTCATGAAAAT 3′ (Supporting Information S1: Table S1). The amplicon was sequenced using the reverse primer. The PCR amplification reaction was as follows: one cycle of 96°C for 5 min, 40 cycles of 96°C for 30 s, 59°C for 30 s and 72°C for 80 s, and one cycle of 72°C for 7 min with a final hold at 4°C.

2.3 | PHS assessment

PHS assessment was conducted on five primary heads from each plot, harvested at physiological maturity from the 2016 and 2017 spring bread wheat yield trials, as well as five primary heads from the 2022 durum PHS trials at physiological maturity and 1-week past physiological maturity. Physiological maturity was defined by the point at which ~80% of the peduncles in a row had lost their green color. Heads were collected into paper bags and stored for 24 h in a forced-air oven set to 37°C. Heads were then stored at −20°C until all rows reached physiological maturity, and heads were collected and dried in each year of experimentation. A misting chamber (Vetch et al., 2019a) was constructed from 2.54 cm diameter PVC pipe, measuring 1.5 meters wide by 4.8 m long by 0.6 m tall. The PVC skeleton was assembled inside a greenhouse with a 16-h photoperiod and average day and night temperatures of 22°C and 18°C, respectively. The chamber was encased in 4 mm clear polyethylene plastic sheeting. The top of the chamber was covered with a 2.54 cm thick reflective foam board to reduce solar heating inside the chamber. Two wire mesh with 0.63 cm^2 boxes were held 7.5 cm apart in a wooden frame and placed in the bottom of the chamber so the harvested wheat heads could be placed

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upright in the chamber, with the bottom of the cut stem held by the lower mesh and the head rested in the upper mesh. The misting system consisted of 3 Rain Bird full circle mist heads with a flow rate of 1.1 gpm, set in the center of the chamber on 15 cm risers and spaced 1 m apart. An Irritrol 700B-75 Ultra Flow NPT Threaded Valve with Flow Control (Irritrol) paired with a Galcon 8056 AC-6S irrigation controller (Galcon USA LTD) was utilized to control the flow rate and sprinkler times, with the chamber being set to spray water for a minute every 5 min. Once all heads were collected and dried for 24 h, for each experimental year, they were immediately placed together in a misting chamber. Wheat heads remained in the misting chamber for a total of 7 days, after which they were assigned a PHS score. Each round of evaluation in the misting chamber took place in September, following the harvest for that year. The PHS scoring system (Vetch et al., 2019a) defines one as completely dormant (showing no visible sprouting structures), two as <10% sprouted, three as 10%–24% sprouted, four as 25%–49% sprouted, five as 50%–74% sprouted, six as 75%–99% sprouted, and a score of seven representing completely nondormant heads where all seeds have germinated. PHS scores per plot were averaged from the five heads in each plot. PHS assessment in the misting chamber occurred directly following the harvest and drying of all materials collected in each experimental year.

2.4 | Variant analysis

Structural prediction for novel *MFT* alleles was performed using the DNAStar NovaFold AI prediction software. Fold prediction was performed on the *MFT* sequences from both the A (TraesCS3A02G006600.1) and B (TraesCS3B02G010100.1) genome-specific copies in spring bread and durum wheat (Lasergene, 2023). Single residue changes were made at the point of SNP for individual alleles and a fold stability prediction was made by the DNAStar Protean3D software for the effect of the amino acid change on the protein. Predictions are evaluated by a ΔFire score in which a number ranging from −0.5 to 0.5 is predicted to have a neutral effect on protein stability, below −0.5 is predicted to be stabilizing to the protein, and above 0.5 is predicted to be destabilizing to the protein.

2.5 | Statistical analysis

PHS scores from 2016 and 2017 were analyzed together with three replications per line per year. PHS scores from the 2022 durum PHS trials were analyzed with three replications per line at physiological maturity and 1 week after physiological maturity. Differences in PHS by allelic class of *MFT* were analyzed by analysis of variance (ANOVA) and Fisher's Least Significant Difference, utilizing the ANOVA function in the car package (Fox & Weisberg, 2019) and the lmer function in the lme4 package (Bates et al., 2015) in R (R Core Team, 2023). Allele class means and least square differences between groups were obtained using the emmeans (Lenth et al., 2018) and agricolae (Mendiburu & Yaseen, 2020) packages in R.

3 | RESULTS

Five missense mutations were discovered when screening the lines in this study (Table 1). Two SNP in the fourth exon, predicted to create an amino acid change for the A genome copy, were identified in durum wheat, named the *TdMFT-3A1b* allele (Supporting Information S1: Figures S1 and S2). One SNP in the second exon, predicted to create an amino acid change for the A genome copy, was identified in spring bread wheat, named the *TaMFT-3A1c* allele (Supporting Information S1: Figures S7 and S8). There were many SNP and INDELs found in the introns of the *TaMFT-3A1c* allele (Supporting Information S1: Figure S9). The same two SNP, predicted to create amino acid changes, were discovered in spring bread wheat and durum wheat in the second and first exons of the B1

TABLE 1 *MFT* alleles identified in this study.

Mutation	Accession	Species	Allele	Genome	Base pair change	Residue	Position	ΔFire
K81R	OQ729930	*aestivum*	*MFT-3A1c*	A	A/G	Exon 2	+399	+0.31
V119E	KF311061	*turgidum*	*MFT-3A1b*	A	T/A	Exon 4	+2864	+4.08
Y131C	KF311061	*turgidum*	*MFT-3A1b*	A	A/G	Exon 4	+2900	+0.53
C4F	OQ729933/OQ729938	*aestivum/turgidum*	*MFT-3B1c*	B1	G/T	Exon 1	+11	−0.48
W86S	OQ729932/OQ729937	*aestivum/turgidum*	*MFT-3B1b*	B1	G/C	Exon 2	+409	+4.11

Note: ΔFire score between −0.5 and 0.5 predict neutral, below −0.5 predict stabilizing, and above 0.5 predict destabilizing.

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genome copy. These two SNP do not appear together in the same allele. These alleles were named *TaMFT-3B1b*, *TdMFT-3B1b*, *TaMFT-3B1c*, and *TdMFT-3B1c* (Supporting Information S1: Figures S3–S6). Screening the advanced yield trial spring bread wheat population revealed five lines that contained the *TaMFT-3A1a* allele (TraesCS3A02G006600.1), analogous to the *TaPHS1* resistant allele previously published in Vetch et al. (2019a) as well as 12 cultivars that contained the *TaMFT-3A1b* allele (OQ729929), analogous to the *TaPHS1* susceptible allele, also from Vetch et al. (2019a) (Tables 2 and 4). *TaPHS* and *TaMFT* are the same gene and have not been named consistently throughout published studies, as first identified by Liu et al. (2013). Here, the *TaMFT* naming convention is carried forward as it more appropriately describes the gene. A previous study by Vetch et al. (2019a) demonstrated a significant increase in PHS susceptibility for the *TaMFT-3A1b* allele with an average PHS score of 1.4 higher than the *TaMFT-3A1a* allele in hexaploid winter bread wheat. Statistical significance was found between these alleles in this study for spring bread wheat, with the trend following the same pattern as Vetch et al. (2019a), where the *TaMFT-3A1b* allele has an average PHS score 0.68 greater than that of the *TaMFT-3A1a* allele (Table 4). While statistical significance was found between these alleles, the low *n* for the *TaMFT-3A1a* allele found in the study population in conjunction with the study design being a haplotype analysis prohibits drawing strong conclusions as to the impact of this allele on PHS. A novel allele named *TaMFT-3A1c* (OQ729930) was discovered in four cultivars that contained an SNP in the second exon, predicted to change the 81st amino acid in the *MFT-3A* protein from a lysine to an arginine (Tables 2 and 4). NovaFold AI does not predict this amino acid change to be destabilizing to the protein; however, the trend continues toward more PHS susceptibility, with an average PHS score of the *TaMFT-*

TABLE 2 Preharvest sprout scores based on 2016 and 2017 advanced yield trial averages for all lines.

MFT-A, MFT-B1 alleles and PHS scores in spring wheat (Triticum aestivum)				
ID	**PHS 2016**	**PHS 2017**	***MFT-A***	***MFT-B1***
Reeder	2.6 ± 0.85	2.2 ± 0.47	MFT-3Aa	MFT-3B1a
MT 1320	4.6 ± 0.75	4.2 ± 0.80	MFT-3Aa	MFT-3B1a
McNeal	1.4 ± 0.26	2.1 ± 0.24	MFT-3Aa	MFT-3B1b
Alum	3.2 ± 0.92	4.8 ± 0.88	MFT-3Aa	MFT-3B1b
MT 1442	3.3 ± 0.20	5.0 ± 0.57	MFT-3Aa	MFT-3B1b
Brennan	4.6 ± 0.75	2.6 ± 0.33	MFT-3Ab	MFT-3B1a
Thatcher	1.3 ± 0.38	2.0 ± 0.65	MFT-3Ab	MFT-3B1b
MT 1525	2.3 ± 0.93	3.1 ± 0.25	MFT-3Ab	MFT-3B1b
MT 1512	3.2 ± 0.71	4.9 ± 0.42	MFT-3Ab	MFT-3B1b
Egan	4.6 ± 1.54	4.0 ± 0.65	MFT-3Ab	MFT-3B1b
DuClair	4.0 ± 0.59	4.8 ± 0.29	MFT-3Ab	MFT-3B1b
Choteau	3.7 ± 0.69	5.3 ± 0.54	MFT-3Ab	MFT-3B1b
MT 1509	3.4 ± 1.12	6.0 ± 0.05	MFT-3Ab	MFT-3B1b
WB9879CLP	4.4 ± 1.17	5.3 ± 0.30	MFT-3Ab	MFT-3B1b
MT 1542	4.5 ± 0.56	5.2 ± 0.12	MFT-3Ab	MFT-3B1b
MT 1570	5.4 ± 0.94	4.5 ± 0.22	MFT-3Ab	MFT-3B1b
MT 1348	3.5 ± 1.09	3.5 ± 0.75	MFT-3Ab	MFT-3B1c
Vida	2.4 ± 1.20	4.7 ± 1.05	MFT-3Ac	MFT-3B1a
MT 1514	2.8 ± 1.84	5.5 ± 0.24	MFT-3Ac	MFT-3B1b
MT 1455	4.8 ± 1.15	6.2 ± 0.05	MFT-3Ac	MFT-3B1b
MT 1401	5.6 ± 1.07	5.7 ± 0.29	MFT-3Ac	MFT-3B1b

Note: Three irrigated environmental reps in both years. 2016 and 2017 correlation coefficient = 0.55. Sprout scoring scale (1–7): A score of 1 is completely dormant after 7 days of wetting, 2 is <10% sprouted, 3 is 10%–24% sprouted, 4 is 25%–49% sprouted, 5 is 50%–74% sprouted, 6 is 75%–99% sprouted, and a score of seven represents completely nondormant heads that have sprouted 100% and shoots are >5.05 cm.

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3A1c allele being 1.32 higher than that of the *TaMFT-3A1a* allele (Tables 1 and 4). While statistical significance for PHS was found between these alleles, it is worth mentioning again that the low n for *the TaMFT-3A1a* and the *TaMFT-3A1c* alleles in conjunction with the study design being a haplotype analysis prohibits drawing strong conclusions as to the impact of this allele on PHS.

Two novel alleles for *TaMFT-B1* were revealed when screening the spring bread wheat population in comparison to *TaMFT-B1a* (TraesCS3B02G010100.1). *TaMFT-3B1b* (OQ729932) is characterized by a predicted amino acid change at the 86th position in the protein from a tryptophan to a serine (Tables 2 and 4). NovaFold AI predicts this amino acid change to be very destabilizing to the protein. At physiological maturity, the difference in PHS between *TaMFT-3B1a* and *TaMFT-3B1b* was statistically significant, with the *TaMFT-3B1b* allele PHS score averaging 0.63 higher than that of the *TaMFT-3B1a* allele (Tables 1 and 4). The second novel allele discovered in spring bread wheats, named *TaMFT-3B1c* (OQ729933) results from a predicted amino acid change of a cysteine to a phenylalanine at the fourth amino acid position (Tables 2 and 4). NovaFold AI does not predict this amino acid change to be destabilizing to the protein, which appears correct as there is no significant difference between the *TaMFT-3B1c* and *TaMFT-3B1a* allele, nor does there appear to be an apparent trend in the data for one allele or the other with respect to PHS (Tables 1 and 4). There was only a single line found with the TaMFT-3B1c allele so conclusions about this allele cannot be drawn from this study. The population screen revealed four lines with the *TaMFT-3B1a* allele, 16 lines with the *TaMFT-3B1b* allele, and one line with the *TaMFT-3B1c* allele (Tables 2 and 4). The low n for *TaMFT-3B1a* and *TaMFT-3B1c*, in conjunction with the study design being a haplotype analysis, prohibits drawing strong conclusions as to the impact of these alleles on PHS.

In durum wheat, no *TdMFT-3A* polymorphisms were found among the lines studied. All cultivar screened were identical for *TdMFT-3A* but the sequence was different from the one accession of durum published on NCBI for *TdMFT-3A* (Table 3 and Supporting Information S1: Figures S1 and S2). The accession for *TdMFT-3A* in durum on NCBI (KF311061.1) was named *TdMFT-3A1a* and the novel allele discovered in this study was named *TdMFT-3A1b* (OQ729935) (Tables 3 and 4). Differences in these alleles are characterized by a predicted amino acid change of a valine to glutamic acid at the 119th amino acid position which NovaFold AI predicts to be very destabilizing (Table 1). A second predicted amino acid change is present in the *TdMFT-3A1b* allele characterized by a change from a tyrosine to a cysteine at the 131st amino acid position (Table 1). NovaFold AI predicts that this change is slightly destabilizing to the *TdMFT-3A* protein (Table 1). It would be worthwhile to investigate the effect of these novel alleles in durum to determine which allele promotes seed dormancy and which allele increases the tendency for PHS. Durum wheat is generally very dormant, which is reflected in the average low PHS scores for durum lines screened in this study. All lines in this study, screened from around the world, contain the *TdMFT-3A1b* allele therefore it is hypothesized to be the functional *MFT-A* allele that promotes seed dormancy and the *TdMFT-3A1a* allele (KF311061.1) is likely an *MFT-A* mutant allele that may impact PHS susceptibility.

The same three alleles found in spring bread wheat *TaMFT-3B1* were present in durum wheat *TdMFT-3B1*, and all were named the same accordingly (Tables 2 and 3). A correlation coefficient of 0.88 was calculated between PHS scores in durum wheat at physiological maturity and 1-week past physiological maturity. In both PHS assessments taken at physiological maturity and 1-week past physiological maturity, the *TdMFT-3B1b* (OQ729937) allele was revealed to be statistically significant in its impact on PHS, increasing PHS susceptibility by an average of 1.83 as compared to the *TdMFT-3B1a* allele (Table 4). No significant difference in PHS was detected between the *TdMFT-3B1c* (OQ729938) and *TdMFT-3B1a* alleles (Table 4). Of the durum cultivars screened, 27 possessed the *TdMFT-3B1a* allele, 3 the *TdMFT-3B1b* allele, and 2 the *TdMFT-3B1c* allele (Table 3). The low n for *TdMFT-3B1b* and *TdMFT-3B1c*, in conjunction with the study design being a haplotype analysis, prohibits drawing strong conclusions as to the impact of these alleles on PHS.

4 | CONCLUSION

In this study, evidence has been provided that *MFT* homologs in both spring bread wheat and durum wheat impact seed dormancy in the same manner as previously demonstrated in winter bread wheat. Functional copies of *MFT* from the A genome and the B1 copy on the B genome promote seed dormancy and resist PHS. Mutant copies of any of these homologs with predicted deleterious amino acid changes frequently result in increased PHS susceptibility. Throughout spring bread wheat breeding history, there has been some selection effort against seed dormancy, reflected in a predominance of the more PHS-susceptible *TaMFT-3A1b* and *TaMFT-3B1b* alleles in the spring bread wheat population screened as compared to the durum wheat population. Durum wheat is generally more dormant than spring bread wheat, given less selection against

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TABLE 3 All varieties screened contain TdMFT-3A1b allele.

PHS scores and *MFT-B1* alleles in durum wheat (*Triticum turgidum*)			
ID	**PHS @ PM**	**PHS 1 Week Past PM**	***MFT-B1***
3602	1.53 ± 0.50	1.27 ± 0.44	*MFT-B1a*
AC navigator	1.67 ± 0.70	2.00 ± 0.52	*MFT-B1a*
AC pathfinder	1.87 ± 0.50	1.87 ± 0.62	*MFT-B1a*
Alzada	2.27 ± 1.24	3.27 ± 0.77	*MFT-B1a*
Blackbeard	1.13 ± 0.34	1.60 ± 0.61	*MFT-B1a*
Divide	1.47 ± 0.50	1.87 ± 0.62	*MFT-B1a*
Durati	1.73 ± 0.77	1.87 ± 0.50	*MFT-B1a*
ELS 6404-124-1	1.00 ± 0.00	1.33 ± 0.47	*MFT-B1a*
Grano	1.27 ± 0.44	1.53 ± 0.50	*MFT-B1a*
Icaro	2.2 ± 1.17	1.87 ± 0.62	*MFT-B1a*
Joppa	1.13 ± 0.34	2.07 ± 0.85	*MFT-B1a*
Kronos	3.67 ± 1.40	3.53 ± 1.93	*MFT-B1a*
Kyle	1.13 ± 0.34	1.07 ± 0.25	*MFT-B1a*
Lustre	1.87 ± 0.88	3.07 ± 1.06	*MFT-B1a*
Miwok	2.20 ± 0.65	3.00 ± 1.37	*MFT-B1a*
Mountrail	1.20 ± 0.40	2.00 ± 0.89	*MFT-B1a*
MTD18148	1.40 ± 0.61	2.27 ± 0.68	*MFT-B1a*
Perigo Candeal 18	1.20 ± 0.40	1.73 ± 0.68	*MFT-B1a*
Plenty	1.20 ± 0.40	1.13 ± 0.34	*MFT-B1a*
Raska	1.27 ± 0.44	2.53 ± 0.88	*MFT-B1a*
Riveland	1.20 ± 0.40	1.60 ± 0.49	*MFT-B1a*
Strongfield	1.20 ± 0.40	1.27 ± 0.44	*MFT-B1a*
Svevo	4.73 ± 1.18	3.67 ± 0.94	*MFT-B1a*
Tioga	1.20 ± 0.40	1.40 ± 0.49	*MFT-B1a*
Valdur	1.07 ± 0.25	1.27 ± 0.44	*MFT-B1a*
Wakooma	1.20 ± 0.40	1.07 ± 0.25	*MFT-B1a*
Wallaori	2.53 ± 1.41	2.20 ± 0.65	*MFT-B1a*
Desert Gold	1.73 ± 0.68	3.07 ± 0.77	*MFT-B1b*
Gaza	1.93 ± 0.85	2.00 ± 0.73	*MFT-B1b*
Kamilaroi	6.47 ± 1.31	6.27 ± 0.77	*MFT-B1b*
Akmamenka	1.13 ± 0.34	1.13 ± 0.34	*MFT-B1c*
MTD112219	2.67 ± 0.87	1.93 ± 0.68	*MFT-B1c*

Note: 2022 field season in three irrigated environmental reps. PM and 1 week past PM correlation coefficient = 0.88. Sprout scoring scale (1–7): A score of 1 is completely dormant after 7 days of wetting, 2 is <10% sprouted, 3 is 10%–24% sprouted, 4 is 25%–49% sprouted, 5 is 50%–74% sprouted, 6 is 75%–99% sprouted, and a score of 7 represents completely nondormant heads that have sprouted 100% and shoots are >5.05 cm. Abbreviations: PHS, preharvest sprout; PM, physiological maturity.

seed dormancy over its breeding history, which is reflected by most durum kinds of wheat screened in this study possessing the *TdMFT-3B1a* allele. While durum wheat was shown to be less PHS-susceptible than spring bread wheat, this study does reveal a range of PHS scores among durum wheat lines. PHS-susceptible alleles of *MFT* are not prevalent in durum lines, but they do exist and may be incorporated into

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TABLE 4 PHS scores by allele compared with analysis of variance.

Haplotype analysis table						
Species	**Allele**	***n***	**PHS average**	**SE**	**LSD**	***p*-Value**
Triticum aestivum @ PM	*MFT-3A1a*	5	3.30	0.27	b	0.006
	MFT-3A1b	12	3.98	0.17	a	
	MFT-3A1c	4	4.62	0.31	a	
	MFT-3B1a	4	3.37	0.32	b	0.095
	MFT-3B1b	16	4.10	0.15	a	
	MFT-3B1c	1	3.47	0.61	ab	
Triticum turgidum @ PM	*MFT-3B1a*	27	1.66	0.06	b	2.20E-16
	MFT-3B1b	3	3.38	0.19	a	
	MFT-3B1c	2	1.90	0.23	b	
Triticum turgidum 1 week after PM	*MFT-3B1a*	27	1.95	0.05	b	2.20E-16
	MFT-3B1b	3	3.78	0.17	a	
	MFT-3B1c	2	1.53	0.21	b	

Note: Means followed by the same letter are not significantly different at the 5% level based on Fisher's LSD. PHS, preharvest sprout; PM, physiological maturity.

cultivars to be released if attention is not paid to *MFT* when breeding. This study revealed novel alleles of *MFT* in both spring bread wheat and durum wheat and provides some evidence of their impact on PHS through a haplotype analysis. This study, being a haplotype analysis with a low n for novel alleles discovered, prohibits drawing strong conclusions as to the impact of these alleles on PHS. A follow-up study comparing these novel alleles in near-isogenic or recombinant inbreed substitution lines is necessary to prove the impact of these alleles on PHS. The falling number can be utilized as an analog for PHS assessment in subsequent studies and may provide a more accurate assessment of dormancy as compared to the visual sprouting test preformed in this study. The information in this study can serve as a resource for spring bread wheat and durum wheat breeders to make selections for alleles of *MFT* that impact susceptibility to PHS.

AUTHOR CONTRIBUTIONS

Brandon J. Tillett carried out field experiments, PHS assessment, genotyping, drafted the text, and constructed the figures and tables. Justin M. Vetch carried out field experiments and PHS assessment. John M. Martin contributed and edited the note. Michael J. Giroux provided supervision, contributed to, and edited the note. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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