Received: 30 November 2024 | Accepted: 25 August 2025

DOI: 10.1002/csc2.70169

Crop Science

ISSUES

Special Section: 15th International Symposium on Pre-Harvest Sprouting in Cereals

Association mapping of preharvest sprouting tolerance in spring wheat reveals genetic connections to late maturity alpha-amylase and vivipary

Scott W. Carle[1] | **Sarah R. Peery**[1] | **Kimberly A. Garland-Campbell**[1,2] | **Michael O. Pumphrey**[1] | **Camille M. Steber**[1,2]

[1]Department of Crop and Soil Sciences, Washington State University, Pullman, Washington, USA

[2]USDA-ARS Wheat Health, Genetics and Quality Unit, Washington State University, Pullman, Washington, USA

Correspondence
Camille M. Steber, USDA-ARS Wheat Health, Genetics, and Quality Research Unit, P.O. Box 646420, Clark Hall, Washington State University, Pullman, WA 99164-6420.
Email: camille.steber@usda.gov

Assigned to Associate Editor Esten Mason.

Funding information
USDA-ARS; Washington Grain Commission

Abstract

Preharvest sprouting (PHS), the initiation of mature grain germination on the mother plant when wheat (*Triticum aestivum* L.) gets wet before harvest, is a major cause of elevated post-harvest alpha-amylase in wheat grain. Alpha-amylase digests starch to support seedling growth during germination. However, excessive starch digestion by alpha-amylase reduces its pasting capacity in flour/water mixtures leading to collapsed cakes, sticky noodles, and bread with sticky crumb. Elevated alpha-amylase can also result from cool temperatures during grain filling, either due to a developmental problem called late maturity alpha-amylase (LMA) or premature germination under moist conditions termed vivipary. PHS tolerance was mapped to 53 quantitative trait loci (QTL), including 16 of high significance (logarithm of the odds > 7.5), in a spring wheat panel previously used for association mapping of LMA. When vivipary assays were performed on a panel subset, vivipary, LMA, and PHS were significantly correlated with each other. This is interesting given that the PHS and vivipary phenotypes assayed were visible germination, whereas the LMA phenotype assayed was alpha-amylase activity. While there are LMA susceptible lines that are PHS tolerant and vice versa, this suggests that overlapping genetic mechanisms may govern tolerance in this population. Indeed, Five PHS QTL had a significant effect of LMA phenotype in the same population *Qs2-1A*, *Qs20-3B*, *Qs31-5A*, *Qs39-5D*, and *Qs42-6A*. Breeding programs may be able to use such QTL to select for both PHS and LMA tolerance. The more significant and reproducible PHS QTL identified are good candidates for marker development and cloning efforts.

Abbreviations: ABA, abscisic acid; ANOVA, analysis of variance; BLUP, best linear unbiased predictors; dpa, days post-anthesis; GA, gibberellin A; GI, germination index; GWAS, genome-wide association study; LMA, latematurity alpha-amylase; LOD, logarithm of the odds; PHS, preharvest sprouting; QTL, quantitative trait loci; SI, sprouting index; SNP, single nucleotide polymorphism; TCAP, *Triticeae* coordinated agricultural project; Ts, time to sprouting score threshold.

Scott W. Carle and Sarah R. Peery contributed equally to this work.

Plain Language Summary

Wheat farmers may be forced to sell their crop at a low price if the grain contains too much alpha-amylase. This enzyme degrades starch causing fallen cakes, sticky bread, and sticky noodles. This can be caused by (1) untimely rain causing preharvest sprouting (PHS) or germination of mature grain on the mother plant, or (2) cool temperatures during grain filling causing the developmental problem late maturity alpha-amylase (LMA). Breeding wheat with genetic resistance can reduce the risk from weather. To help achieve this, PHS resistance genes were mapped to 16 chromosome locations. When the PHS data were compared to LMA data, there was enough agreement to suggest that they are sometimes genetically related problems. Three of the PHS genes were located close enough to LMA genes to suggest they may be the same or neighboring genes. Selection for single and multi-purpose loci/genes may speed up breeding for resistance to these weather-triggered problems.

1 | INTRODUCTION

Wheat (*Triticum aestivum* L.) farmers receive lower prices for their grain when it contains too much α-amylase enzyme activity, detected in the wheat industry using the falling number method (reviewed in Ross and Bettge [2009]). The wheat grain or caryopsis is both the means of propagation and the product milled into flour that is used to make diverse products, including bread, crackers, cookies, cakes, and noodles. Elevated α-amylase in wheat grain digests starch in flour/water mixtures, reducing its gelling capacity and leading to cakes that fall, and sticky bread and noodles. Breeding for tolerance to elevated α-amylase at harvest is complicated by the fact that it is controlled by multiple genetic and environmental causes. This study aimed to improve breeding by identifying loci contributing to the main cause of elevated α-amylase, preharvest sprouting (PHS), and by examining genetic connections to two other causes, vivipary and late maturity alpha-amylase (LMA) (reviewed in Derera [1989], Mares and Mrva [2014], Peery et al. [2023], and Sjoberg et al. [2020]).

Alpha-amylase expression in wheat grain can be induced by environmental conditions before or after the completion of grain development. PHS is the initiation of mature grain germination on the mother plant when rain occurs before harvest (Zadoks stage 91; Zadocks et al., 1974). Vivipary in wheat is the precocious germination of immature grain (Zadoks 85), and is promoted by cool and moist conditions during grain maturation (Derera, 1989; Ichinose et al., 2002; Peery et al., 2023). *Viviparous* (*vp*) mutants of maize (*Zea mays* L.) also initiate germination during the maturation stage of kernel development (McCarty, 1995). Vivipary differs from PHS in that the grain never completes the maturation processes of becoming dormant, acquiring desiccation tolerance, and storing nutrients from the mother plant. LMA is the induction of α-amylase by non-freezing cold temperatures during the maturation phase of grain development (Zadoks stage 85; A. P. Derkx & Mares, 2020; Mrva et al., 2006). LMA occurs without visible germination in response to cool temperatures when conditions are not humid (Peery et al., 2023).

Grain dormancy can account for 60 to 85% of PHS tolerance (DePauw & McCaig, 1991), while much of the remaining tolerance can result from differences in spike morphology (King & Richards, 1984; King & von Wettstein-Knowles, 2000). The seeds of many temperate species have dormancy at maturity, resulting in an inability to germinate under favorable conditions until exposed to dormancy-breaking conditions (reviewed by Finkelstein et al. [2008]). Seeds acquire dormancy and desiccation tolerance in response to the hormone abscisic acid (ABA) produced during the maturation phase of seed development. Higher wheat dormancy is associated with higher ABA hormone levels and sensitivity to ABA-inhibition of seed germination (Martinez et al., 2016; Tuttle et al., 2015; Walker-Simmons, 1987; Xu et al., 2016). Dormancy loss and germination are associated with higher gibberellin A (GA) hormone levels and sensitivity. Wheat grain dormancy is strongest at physiological maturity, and then lost over time through dry after-ripening (Schramm et al., 2010). Wheat grain dormancy is also lifted by cold stratification when incubated under cool and moist conditions (Tuttle et al., 2015; Xu et al., 2016). Breeding wheat that has sufficient dormancy at physiological maturity to prevent sprouting when it rains, but not so much dormancy that it will interfere with germination and emergence once those seeds are

planted, is a continual breeding challenge (Rodriguez et al., 2015).

Thus far, there has been little evidence that quantitative trait loci (QTL) for PHS and LMA tolerance overlap or share mechanisms of action, leading to the theory that they are genetically distinct phenomena (D. J. Mares & Mrva, 2014). For example, none of the QTL mapped for LMA in the Cranbrook × Halberd population were linked to PHS QTL mapped in the same population (D. J. Mares & Mrva, 2001; Mrva & Mares, 2001). However, Martinez et al. (2018) identified a QTL for falling number after artificial rain that appeared to co-localize with the LMA QTL identified on chromosome 7B (A. Derkx et al., 2021), raising the possibility that there may be some overlap in genetic mechanisms controlling PHS and LMA. Of the LMA loci mapped thus far, only the strong QTL on chromosome 7B was identified by map-based cloning and has homology to a GA biosynthesis enzyme *ent*-copalyl diphosphate synthase (A. Derkx et al., 2021).

Although QTL associated with PHS tolerance have been found on every wheat chromosome, few QTL for PHS tolerance have been cloned including *Red-1* (*R-1*), *Mother of FT and TFL* (*TaMFT-3A*), and the wheat orthologue of *mitogen-activated protein kinase kinase3* (*TaMKK3-4A*). The dominant *R-1* loci on chromosomes 3A, 3B, and 3D result in additive increases in red pigmentation in the wheat seed coat and in higher dormancy and ABA sensitivity (Himi & Noda, 2005; Himi et al., 2002; Warner et al., 2000). The wheat *TaMFT-3A* gene was identified as a transcript induced in response to dormancy-enhancing cool temperatures during grain maturation, and subsequently found to co-localize with a major QTL for PHS tolerance on chromosome 3A (also called *TaPHS-1*; S. Liu et al., 2013; Nakamura et al., 2011). ABA regulates the MAP kinase cascade that includes *MKK3* in *Arabidopsis thaliana* (L.) and in rice, *Oryza sativa*, and *MKK3* acts as a negative regulator of dormancy (Danquah et al., 2015; Mao et al., 2019; Xi et al., 2010). A major QTL for PHS tolerance mapped in a wide range of germplasm was shown to be the wheat ortholog, *TaMKK3-4A* (also called *Phs-A1*; Shorinola et al., 2017; Torada et al., 2016). To avoid confusion, we refer to these genes as *TaMFT-3A* and *TaMKK3-4A*.

To identify PHS QTL and to determine whether they co-localize with QTL for LMA, we performed a genome-wide association study (GWAS) for PHS tolerance using visible sprouting in spike-wetting tests in an association panel of North American spring wheat in which we previously mapped seven QTL for LMA tolerance (C. Liu, Parveen et al., 2021). While visible sprouting is not always correlated to falling number or α-amylase levels, it is well correlated in stronger sprouting events (Brown et al., 2018; Depauw & McCaig, 1991; Martinez et al., 2018). To improve PHS mapping, the trait was mapped based not only on degree of visible sprouting on a specific day of misting, but also on the time required to reach a given score. Comparisons with the previous study revealed co-localization of two PHS and LMA loci, and analysis showed significant correlations between PHS, vivipary, and LMA in a subset of this population.

Core Ideas

- A genome-wide study found 53 quantitative trait loci (QTL) associated with preharvest sprouting (PHS) tolerance in spring wheat.
- The major PHS QTL *Qs42-6A* was reproducibly detected on chromosome 6A.
- Tolerance to PHS, vivipary, and LMA were moderately correlated in this population.
- Five PHS QTL also provided LMA tolerance in the same population, suggesting some shared genetic mechanisms.

2 | MATERIALS AND METHODS

2.1 | Germplasm

This study evaluated PHS and vivipary in the association panel of 251 hard red and soft white spring wheat lines assembled by the *Triticeae* coordinated agricultural projects (TCAP, https://www.triticeaecap.org). This panel was previously characterized for LMA susceptibility in the field and greenhouse (C. Liu, Parveen, et al., 2021), root morphology, stem and stripe rust susceptibility, Hessian fly susceptibility, and agronomic traits (Bajgain et al., 2015; Godoy et al., 2018; Narayanan & Vara Prasad, 2014; Prather et al., 2022). The panel is comprised of lines from 10 North American Breeding programs. Spring cultivars included as controls based on previous characterization for LMA and vivipary (Table S1; Peery et al., 2023) were Maringa (PI584924; Barrero et al., 2013), Seri-82 (PI591774; Tan, 2004), Halberd (PI377885; Mrva & Mares, 2001), Spica (PI213830; Barrero et al., 2013), WB6341 (Clark, 2015), and the Washington State University spring breeding line WA8124 (IDO599/S2K00095; C. Liu, Tuttle, et al., 2021). Four winter wheat lines were included as controls to verify even misting during spike-wetting tests: PHS-tolerant lines, soft white Brevor (CI12385; Heyne, 1959) and hard white Clarks Cream (PI 476305; Graybosch et al., 2019); and PHS-susceptible Bruneau (PI 664304, PVP201200014) and hard red Greer (AgriPro) (Martinez et al., 2018; Tuttle et al., 2015; Walker-Simmons, 1987).

2.2 | Vivipary germination assays of immature grain

A total of 37 TCAP lines were characterized for vivipary, including 19 LMA-resistant lines and 18 LMA-susceptible lines chosen based on LMA phenotypes in C. Liu, Parveen, et al. (2021) (Table S1). A subset of the TCAP population was used because the assay is labor- and resource-intensive. Vivipary was assayed by characterizing the germination of immature kernels at the soft dough stage of grain filling (as in Peery et al. [2023]). Mother plants used to obtain kernels for vivipary experiments were vernalized for 2 weeks at 4°C to synchronize flowering and grown under a 16-h day and 8-h night photoperiod (Conviron GR96, 300 $\mu mol^{-1}\ m^{-2}\ s^{-1}$) with a 23°C–25°C day and 17°C–18°C night. To obtain grain at the soft dough stage of grain maturation, spikes were tagged at anthesis, and spikes were harvested at 24–27 days post-anthesis (dpa) at the soft dough stage (Zadoks 85) of grain maturation previously found to be optimal for inducing wheat vivipary (Peery et al., 2023). The goal was to have a minimum of three biological replicates per treatment temperature, consisting of a minimum of one spike from each of three plants, where each spike of 20–30 kernels was plated on a separate Petri dish. A total of 6–12 spikes from three to six plants per genotype were harvested and hand-threshed into a 15-mL tube containing a Kimwipe moistened with sterile water. Three to six biological replicates (three to six spikes on three to six Petri plates) were plated at the warm (25°C day/18°C night) and three to six were plated at the cool (18°C day/7.5°C night) incubation temperature. For each spike, 20–30 kernels were sterilized in 10% bleach/0.01% sodium dodecyl sulfate, washed six times with sterile water, then plated on separate 0.5x Murashige and Skoog (MS) medium/0.08% agar plates. Plates were incubated in a diurnal growth chamber (Percival Models #E30B and #CU22L) with a 16-h day/8-h night (178 $\mu mol^{-1}\ m^{-2}\ s^{-1}$). Germination was scored daily for 7 days, and percent germination was calculated daily. Germination index (GI) was calculated over 7 days of imbibition as an index where greater weight is given to germination on an earlier day of incubation as follows:

$$GI = \frac{7 \bullet G_{day1} + 6 \bullet G_{day2} + 5 \bullet G_{day3} + 4 \bullet G_{day4} + 3 \bullet G_{day5} + 2 \bullet G_{day6} + G_{day7}}{(7 \bullet n)}$$

where GI is the germination index, G_{day} is the number of seeds newly germinated on the indicated day, and n is the total number of kernels incubated on the Petri dish (Martinez et al., 2016). The highest possible GI is 1.0 when all kernels germinate on day 1.

2.3 | Spike-wetting tests

Wheat for spike-wetting tests was grown at the Washington State University Spillman Agronomy Farm in Pullman, WA, in 1.5-m long headrows in 2019 and 2020 using the agronomic practices recommended for the region (Schillinger et al., 2006). There were two complete field replications for each genotype grown per year. Some lines had additional replication, and some lines had only one field replication successfully harvested at physiological maturity in 2019 or 2020. Spike-wetting tests were performed on 226 of the 251 TCAP lines in 2019 and on 250 of the 251 lines in 2020. One spike each of the four internal controls, Brevor, Clark's Cream, Bruneau, and Greer, were placed in every tray as a spatial control for the misting system. Spike-wetting tests were performed as described in Martinez et al. (2018). Wheat spikes were harvested from the field at physiological maturity. Physiological maturity is at the end of grain filling when wheat grain reaches its maximum dry weight and dormancy. Spikes were allowed to after-ripen at room temperature for 5 days, then stored at −20°C to reduce dormancy loss through after-ripening and kill insects (Paterson et al., 1989). Five spikes were misted for each field replicate of each genotype under a greenhouse misting system providing 6 s misting every 60 s. Spikes were scored daily for 7 days for the appearance of visible sprouting based on a 1–10 scale (Table S2; derived from McMaster and Derera [1976]). The effects of both grain dormancy and spike morphology contribute to the measured data. The spike-wetting test data collected consisted of sprouting scores after 1, 2, 3, 4, 5, 6, and 7 days of misting. The sprouting index (SI) was calculated as a summary statistic over the 7 days as follows:

$$\mathrm{SI} = \frac{7\left(\mathrm{S_{d1}}\right) + 6\left(S_{\mathrm{d2}} - S_{\mathrm{d1}}\right) + 5\left(S_{\mathrm{d3}} - S_{\mathrm{d2}}\right) + 4\left(S_{\mathrm{d4}} - S_{\mathrm{d3}}\right) + 3\left(S_{\mathrm{d5}} - S_{\mathrm{d4}}\right) + 2\left(S_{\mathrm{d6}} - S_{\mathrm{d5}}\right) + \left(S_{\mathrm{d7}} - S_{\mathrm{d6}}\right)}{(\text{number of days scored} \bullet n)}$$

where S is the sprouting score given for the day (d) of misting and n is the maximum possible score of 10. The SI approximates the area under the sprouting curve, giving more weight to earlier sprouting. The minimum SI is 0.1 if there is no germination, and the maximum is 1.0 if all spikes reach a score of 10 on day 1 of misting.

2.4 | Temporal analysis of sprouting score thresholds by linear regression

The time required to reach specific sprouting scores was calculated as a trait for mapping. Linear regression was performed on sprouting score observations (y) versus time in days (x) for each individual spike. In order to prevent redundant scores at the minimum (1) and maximum (10) of the scale from exerting undue influence on these regressions, filtering was performed to remove all the minimum scores prior to the last recorded minimum score for that spike, and all the maximum scores after the first maximum score for that spike. Thus, regressions were performed for individual spikes over the range of time when the sprouting scores were increasing. The linear regression for each wheat spike and the data points that generated each were then put through an alignment process to synchronize the results from all wheat spikes in the dataset to the same relative stage in the progression of scores from 1 to 10; the points in time at which these regressions passed through given threshold sprouting scores were calculated and used as the time to sprouting score threshold (Ts) values for mapping. The linear regression algorithm was used to interpolate or extrapolate the time in days needed for each spike to cross a sprouting score threshold, where the threshold values were sprouting scores of 1.1, 1.5, 2.5, 3.5, 5.5, and 7.5. The calculated regressions omitted some of the most extreme data with slopes of 0, where no change in germination stage could be calculated because insufficient germination occurred. These data were then averaged by genotype within each year. This averaging was performed to maintain the average slope of increasing score for each individual spike because a linear regression performed over the data from all spikes as a lump sum would have flattened as it minimized the square of score-time distances.

2.5 | Statistical analyses

Statistical analyses were performed in RStudio, using R version 4.0.5 (R Core Team, 2024; RStudio Team, 2019). Analysis of variance (ANOVA) was performed using the aov command in the stats package in R. Significant sources of variation were included as covariates when modeling best linear unbiased predictors (BLUPs) (Henderson, 1975). The initial sprouting data were not normal.

To obtain normal PHS trait distributions for use in association mapping, cube root, square root, and $\log_{10}$ transformations were examined in conjunction with BLUP calculations. The cube root transformation was selected based on comparison of Akaike information criterion values and applied prior to BLUP calculation for each sprouting score by day and SI trait based on a mixed linear model using the lmer function from the LME4 package for R (Bates et al., 2015). Factors that showed significant effects in an ANOVA, genotype, week, and tray-nested-within-year were used as covariates in the model to calculate BLUPs (Table S3). Year also had significant variation in the ANOVA, but was omitted from the model to prevent overparameterization. BLUPs were calculated for 2019 and 2020 data, and for the 2 years combined ("both" in Table 1). Vivipary plating assays generated percent germination on days 1 through 7 and GI from assays performed at the warm or cool incubation temperature. In calculating GI BLUPs, genotype was used as a random effect. Based on ANOVA, replication was used as a covariate for cool but not warm GI BLUP calculation. LMA BLUPs for the TCAP population were as previously published for greenhouse LMA induction experiments performed in 2018 and 2019, as were phenotypic categories based on the number of times LMA was induced in eight replications (C. Liu, Parveen, et al., 2021).

2.6 | Genome-wide association study

Previous work characterized 19,190 single nucleotide polymorphisms (SNPs) polymorphic in 247 TCAP lines genotyped using the Illumina iSelect 90K wheat SNP array (Bajgain et al., 2015; S. Wang et al., 2014). The physical location of SNPs mapped is based upon the wheat genome

TABLE 1 Preharvest sprouting QTL identified in the TCAP.

Locus	Chr[a]	Marker	Max LOD[b]	Traits[c]	Years	M[d]	MAF	Tol/Sus[e]
Qs1-1A[f]	1A	IWB45874	8.4	Ts5.5, Ts7.5, d5	19, Both	F, B	0.159	**C**/T
	1A	IWB31350	6.0	d7	Both	F	0.255	**G**/A
	1A	IWB10042	6.8	d4	Both	B	0.158	**C**/T
	1A	IWB5360	6.1	d3	Both	F	0.095	**T**/C
Qs2-1A	1A	IWB9474	8.6	Ts3.5, Ts5.5, d5, SI	20, Both	F, B	0.342	**C**/T
	1A	IWB35143	5.5	d6	20	F	0.256	**A**/G
	1A	IWB34851	11.2	Ts5.5, d4	20, Both	F, B	0.494	**G**/A
	1A	IWB57894	5.5	d4	Both	B	0.271	**C**/T
	1A	IWB35476	6.4	Ts1.1	Both	B	0.322	**C**/T
	1A	IWB45352	8.9	d3, d4	Both	F, B	0.136	**A**/G
Qs15-2B	2B	IWB44316	11.2	Ts5.5, d4	19, Both	F, B	0.309	**C**/T
Qs17-2D	2D	IWB39211	8.8	Ts3.5, Ts7.5	19	F	0.189	**T**/C
	2D	IWB60517	5.5	Ts3.5	Both	B	0.202	**C**/A
Qs18-3A	3A	IWB38315	7.6	Ts3.5, Ts5.5, Ts7.5, d6, d7	19, 20, Both	F, B	0.368	**C**/A
Qs20-3B	3B	IWB65401	8.4	d6, SI	20	F	0.074	**T**/C
	3B	IWB6485	6.3	d5	19	B	0.363	**G**/A
Qs24-4A	4A	IWB275	7.6	Ts1.1, Ts1.5	20	B	0.482	**A**/G
	4A	IWB9276	7.8	Ts_2.5, Ts_3.5, Ts_5.5, Ts_7.5	Both	F	0.482	**T**/C
Qs31-5A	5A	IWB12023	10.3	Ts1.5, Ts2.5, Ts3.5, Ts5.5, Ts7.5, d3	20, Both	F, B	0.284	**C**/T
Qs35-5B	5B	IWB20439	12.8	Ts3.5, d4, d5	20, Both	F, B	0.496	**G**/T
	5B	IWB6830	10.0	Ts2.5, Ts5.5, d5, d6, SI	20, Both	F, B	0.484	**C**/A
	5B	IWB5738	9.1	d7	Both	F, B	0.478	**A**/G
Qs39-5D	5D	IWB6788	8.7	Ts2.5, Ts5.5, Ts7.5, d5, d6, d7, SI	20, Both	F, B	0.445	**A**/G
	5D	IWB40957	8.1	Ts1.1, Ts1.5, Ts2.5, Ts3.5, Ts5.5, d5, d6, d7	19, Both	F, B	0.438	**A**/C
Qs42-6A	6A	IWB47346	12.9	Ts1.1, Ts1.5, Ts2.5, Ts3.5, Ts5.5, d4, d5, d6, d7, SI	19, Both	F, B	0.307	**G**/A
	6A	IWB30050	9.2	Ts1.1, Ts1.5, Ts2.5, Ts5.5, Ts7.5, d4, d5, d6, SI	20, Both	F, B	0.328	**A**/G
Qs44-6B	6B	IWB47211	8.2	d3	19,20, Both	F, B	0.089	**T**/C
	6B	IWB67439	6.5	Ts1.1, Ts1.5, d5	20, Both	F	0.128	**A**/C
	6B	IWB69753	6.7	Ts7.5	Both	F, B	0.132	**T**/C
Qs48-7A	7A	IWB5820	9.3	d3	19	B	0.072	**G**/A
Qs50-7A	7A	IWB4830	6.6	Ts3.5	20, Both	B	0.296	**T**/G
	7A	IWB48466	7.9	d3	20	F, B	0.391	**T**/C
Qs53-7D	7D	IWB53131	5.7	Ts3.5	19	F	0.368	**T**/C
	7D	IWB63737	11.2	Ts1.1, Ts1.5, Ts2.5, d5	Both	F, B	0.462	**C**/T
	7D	IWB19259	9.1	Ts2.5, Ts3.5, Ts5.5, Ts7.5, d5, d6, d7, SI	19, Both	F, B	0.459	**G**/A

Abbreviations: BLINK, Bayesian-information and linkage-disequilibrium iteratively nested keyway; FarmCPU, Fixed and random model Circulating Probability Unification; MAF, minor allele frequency; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; TCAP, *Triticeae* coordinated agricultural project.

[a]Wheat chromosome.

[b]Maximum logarithm of the odds (LOD) among all of the traits mapped to a given SNP marker. The complete list of LOD can be found in Table S4.

[c]Traits, where, d represents day, whereby d3 means the sprouting score on day 3. Ts represents the time needed to reach a threshold of sprouting score, whereby Ts2.5 means the time it took a genotype to pass a sprouting score of 2.5 determined by linear regression. SI is the sprouting index.

[d]M is the GWAS method, B is BLINK, and F is FarmCPU.

[e]Tol (tolerant) and Sus (susceptible) alleles. The first nucleotide (bolded) is the allele variant that shows less or slower sprouting in a spike-wetting test.

[f]Qs is a designation for sprouting QTL.

sequence version 2.0 (Appels et al., 2018). Marker associations with spike-wetting test traits were calculated by GWAS using the fixed and random model circulating probability unification (FarmCPU) and Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) algorithms (Huang et al., 2019; X. Liu et al., 2016) performed in the Genome Association and Prediction Integrated Tool (GAPIT) v3.1 package in R (J. Wang & Zhang, 2021). Default parameters were used for both algorithms. A principal component analysis was performed in GAPIT upon the genotyping data and used as a factor in the GWAS. SNPs with a minor allele frequency below 0.05 were excluded to reduce false positives. Associations that exceeded the LOD (logarithm of the odds) threshold of 5.5 were considered significant.

3 | RESULTS AND DISCUSSION

3.1 | Analysis of PHS tolerance by spike-wetting test

PHS tolerance was characterized using spike-wetting tests of the spring TCAP panel previously characterized for LMA (Liu, Parveen et al., 2021). To obtain sprouting scores by day, spikes placed under a greenhouse misting system were scored daily for 7 days using a 1–10 sprouting scale, where a higher score indicates more advanced sprouting (Table S2; Martinez et al., 2018; McMaster & Derera, 1976). A sprouting score of 5 represents uniform germination over the spike, and scores above 5 represent increasing seedling growth. No sprouting was observed on days 1 and 2 of misting. Based on sprouting scores from days 3 through 7, the population showed stronger dormancy in 2019 than in 2020 (Figure 1a). Sprouting scores ranging from 1 to 10 were reached in 5–6 days of misting. SI was calculated as a summary statistic. A cube-root transformation was applied before calculating BLUPs because SI and sprouting score were not normally distributed (Figure 1b,c). Although there was more dormancy in 2019 than in 2020, spike-wetting test data were highly correlated between years, indicating that it was valid to map using BLUPs calculated from combined 2019 and 2020 data (Figure 1d, for SI, $r = 0.78$; Figure S1A–E). Sprouting scores were more highly correlated between 2019 and 2020 at days 4, 5, 6, and 7 of misting ($r = 0.72$–0.76) than for day 3 ($r = 0.58$) (Figure S1A–E).

While BLUPs of sprouting scores and SI have been used to map, both metrics have limitations (P. Kulwal et al., 2012; Martinez et al., 2018). Sprouting scores by day provide a snapshot on a given day, but do not fully describe the changes in score over time. While SI partly addresses this by integrating sprouting data over time, some information is lost as the

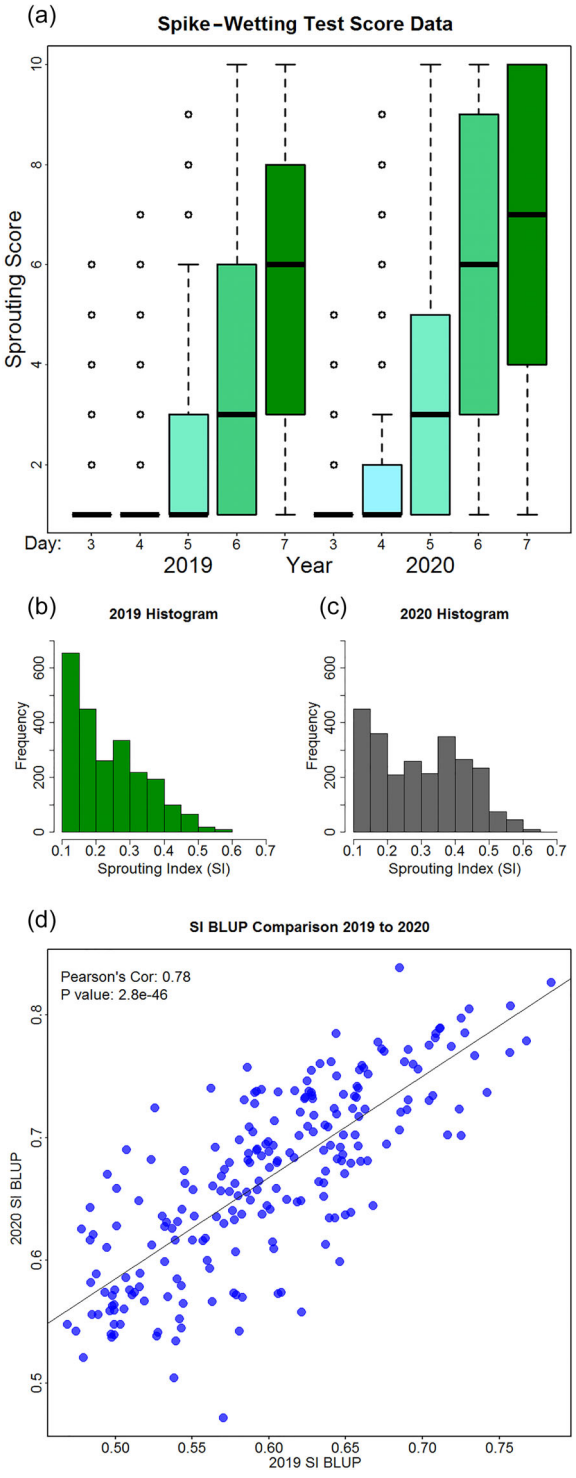

FIGURE 1 Distributions of preharvest sprouting (PHS) traits from spike-wetting tests of the spring *Triticeae* coordinated agricultural project (TCAP). (a) Sprouting score on each day of misting from 2019 and 2020. Spike-wetting tests were performed using five spikes per line/replicate and two field replications per genotype per year. (b) 2019 and (c) 2020 histograms of sprouting index calculated over 7 days of misting. (d) Comparison of cube-root best linear unbiased predictors (BLUPs) for sprouting index (SI) from the field years 2019 and 2020 showed a significant correlation ($r = 0.78$, $p = 2.8 \times 10^{-46}$).

data are compressed. To address these limitations, mapping was also performed based on the time needed for a spike of a given variety to reach a threshold sprouting score (Ts). The sprouting thresholds chosen included the time needed for germination initiation (sprouting score Ts1.1, Ts1.5), progression (Ts2.5, Ts3.5), germination completion (Ts5.5), and seedling growth (Ts7.5). The data from single spikes were analyzed by linear regression, and intercepts were used to determine the days required to reach each score threshold (Figure 2a). Threshold data were averaged over all spikes for each variety to generate traits for mapping.

Graphical representations of the Ts data were generated to examine the validity of the approach. When examined per spike, the linear regressions performed on this dataset produced many different slopes for scores over time and displacements in time (Figure 2a). After transformation from time to percent completion, the linear regressions performed on each spike (when combined as a whole) conformed to a linear model, suggesting that this is a valid model to extrapolate the time required to reach threshold sprouting scores (Figure 2b).

3.2 | GWAS for PHS tolerance

A GWAS was conducted using spike-wetting test data to identify PHS QTL in the North American spring wheat lines comprising the TCAP. PHS tolerance was mapped based on 12 traits: (1) BLUPs of sprouting scores after 3, 4, 5, 6, and 7 days of misting, (2) BLUPs of SI over 7 days of misting, and (3) the time required to reach sprouting thresholds (Ts1.1, 1.5, 2.5, 3.5, 5.5, 7.5). BLUPs were calculated for 2019, 2020, and for both years combined. When GWAS using both FarmCPU and BLINK algorithms (Huang et al., 2019; X. Liu et al., 2016), a total of 263 marker-trait associations with a LOD over 5.5 were detected, representing 53 QTL on 20 chromosomes based upon the assumption that marker pairs within less than 5 cM or 5 Mbp of each other represent the same locus (Table S4; Maccaferri et al., 2015; S. Wang et al., 2014). Twenty loci were detected by both FarmCPU and BLINK, while 25 were detected by FarmCPU alone and eight by BLINK alone (Figure S2A). Given the large region of suppressed recombination around the wheat centromere, hundreds of millions of base pairs of a chromosome can fall within a single QTL based on cM distances, making it difficult to judge if a locus consists of one or multiple genes. For example, the first locus (*Qs1-1A*) that mapped within the centromeric region of chromosome 1A spanned the 387,590,100 bp distance from IWB45874 to IWB5360 within only 8 cM (S. Wang et al., 2014). We focused our discussion on the highly significant 15 loci with a LOD of 7.5 or higher that were also validated as having a significant effect on the trait by one-way ANOVA (Table 1; Figure S4).

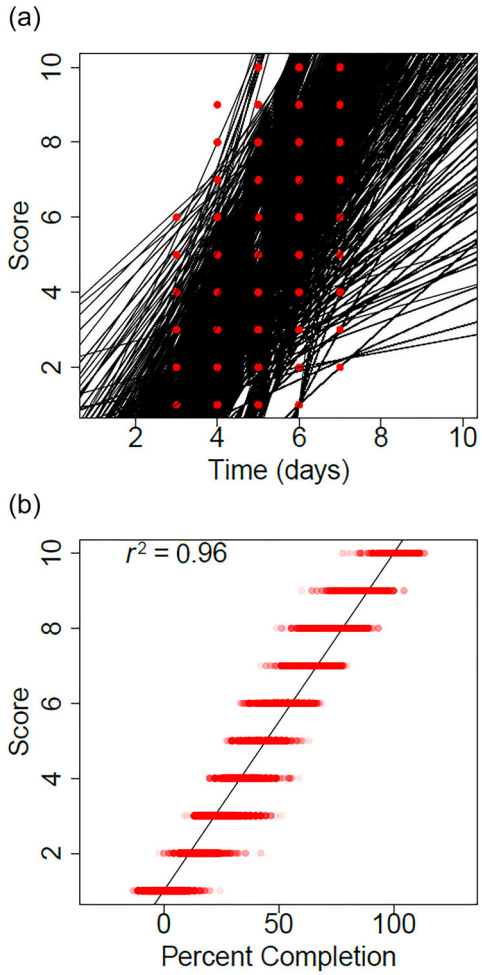

FIGURE 2 Evaluation of the linear regression method for determining the time required to reach sprouting score thresholds (Ts, time to sprouting score). (a) Lines showing the linear regression (black lines) for each spike scored on days 3 through 7 of misting. This is a better way of looking at the sprouting of a spike than the observed scores on each day shown as red dots. The trait used for mapping was the time (in days) at which the regression line intersected with a given sprouting score threshold, for example, a sprouting score of 1.5. Ts was determined for each spike and then averaged for each genotype. (b) To check the validity of the linear model, both the regressions (black lines) and the observations giving rise to them (red) were transformed by converting time to percent completion (where a score of 10 is 100% complete, and a score of 1 is 0% complete). The linear model proved to be a reasonable fit, though the overlap in percent completion at different scores highlights the limited precision of the spike-wetting test scores.

Four loci with LOD over 7.5 were detected with all three year parsings (2019, 2020, and both combined), suggesting a reproducible and strong effect on PHS tolerance, *Qs18-3A*, *Qs39-5D*, *Qs42-6A*, and *Qs44-6B* (Table 1; Table S4; Figure S2B). All of these QTL were detected based on both sprouting score and Ts threshold traits. It should be noted that the effects reported for traits (Table S4) reverse direction

depending on whether the trait is derived of sprouting score (positive effect represents more sprouting), or Ts (positive effect represents taking longer to sprout), but in all cases the more tolerant SNP variant is reported first. The most impressive QTL was *Qs42-6A*, resulting from the association of two SNP markers 2.5 kb apart with every trait–year combination but for day 3 sprouting score with high significance (LOD 5.5–11.2). *Qs42-6A* had a strong effect, resulting in a maximum shift of 0.47 days in time required to reach a sprouting threshold of 7.5. It colocalized with two published PHS QTL (S. Kumar et al., 2015; Zuo et al., 2019). *Qs18-3A* on chromosome 3A (single SNP, LOD 5.8–7.6) was associated mainly with later sprouting traits, including sprouting score on days 6 and 7 and thresholds Ts3.5, 5.5, and 7.5. In contrast, the QTL *Qs44-6B* (three SNPs with LOD 5.5–8.2) was detected mainly by early traits, including sprouting scores on day 3 and 5 of misting and thresholds Ts1.1 and 7.5. *Qs44-6B* colocalized with *QPhs.wsu.6B* identified in Pacific Northwest winter wheat by Martinez et al. (2018). The QTL *Qs39-5D* (two SNPs, LOD of 8.6) was located within 2 cM but 140 Mbp of a wheat homolog of barley seed dormancy gene Q_{SD1} encoding an alanine aminotransferase (Sato et al., 2016).

Several highly significant QTLs were detected in 1 year and/or in both years combined using both threshold and sprouting traits (Table 1; Figure S3), including *Qs2-1A*, *Qs15-2B*, *Qs35-5B*, and *Qs53-7D*. The *Qs2-1A* locus spanned six SNP markers over 7.8 Mbp (LOD 5.5 to 11.2) using three sprouting thresholds, SI, and sprouting scores by day (Table 1). While the *Qs15-2B* locus (LOD 6.0–11.2) was detected with a single SNP based on only two traits (Ts5.5 and d4 sprouting score), it colocalized within 764 kb of the *QPhs.cnl-2B.1* QTL detected with a LOD of 27.1 (Munkvold et al., 2009). Many studies have identified QTL within this region, suggesting a locus providing PHS tolerance in diverse germplasm (P. L. Kulwal et al., 2004; A. Kumar et al., 2009, 2015; Somyong et al., 2014). *Qs35-5B* was highly significant (LOD 5.5–12.8) and colocalized within 11 Mbp of a previous finding of Lin et al. (2015) and within the interval reported by Zanetti et al. (2000). *Qs53-7D* mapped to three SNPs on chromosome 7D using all types of traits, sprouting score, SI, and Ts (LOD of 5.5–11.2) (Figure S3).

When all 53 QTL with LOD > 5.5 were considered, we found that the sprouting threshold trait detected 36 QTL, whereas sprouting scores detected 31 and SI detected nine (Figure 3). There were 16 loci detected by at least two methods, with six being found with all three methods. The six QTL detected using all three traits, sprouting score, SI, and Ts (Figure 3), included *Qs2-1A*, *Qs16-2B*, *Qs35-5B, Qs39-5D*, *Qs42-6A*, and *Qs53-7D*. Some highly significant QTLs (LOD > 7.5) were detected best either with sprouting scores or with Ts. For example, *Qs20-3B* was detected only with sprouting scores in both 2019 and 2020 (d5, d6, SI, LOD 5.9 to 8.4), and *Qs48-7A* was detected only with d3 sprouting scores

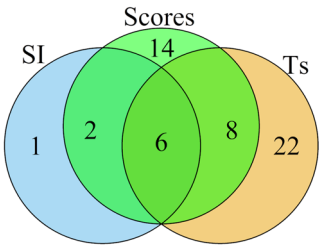

FIGURE 3 Venn diagram of the genome-wide association study (GWAS) loci resulting from the different preharvest sprouting traits calculated from the spike-wetting test data, including sprouting index (SI), the sprouting score on each day of misting, and time to sprouting threshold (Ts). Six loci were detected using all traits, but there were examples of loci uniquely mapped with each trait type.

(LOD 9.3) in 2019 (Table 1). *Qs17-2D* and *Qs24-4A* were all detected only based on threshold traits with high significance (Table 1). *Qs31-5A* was detected based on association of a single SNP with six PHS traits (max. LOD 10.3), of which all but a single trait (d3, LOD 5.6) were Ts threshold traits. It colocalized with previously mapped PHS loci (Lin et al., 2016; Zuo et al., 2019), and had a strong effect, shifting the time to reach a given threshold by as much as 0.51 days of misting. Thus, sprouting scores and sprouting thresholds appear to be complementary approaches that may broaden the spectrum of QTL confidently detected.

3.3 | Loci affecting both PHS and LMA phenotypes

Two PHS QTL were located near LMA QTL previously mapped in the same population *Qs2-1A* and *Qs20-3B* (C. Liu, Parveen, et al., 2021). Interestingly, the *Qs2-1A* PHS locus contains the same SNP (IWB35476, *QLMA.wsu.1A*) mapped for LMA tolerance in the same population with the same favorable allele (C. Liu, Parveen, et al., 2021). Next, we examined the effect of both the PHS QTL and the original LMA QTLs on both the PHS trait SI and on the LMA phenotype (Figure S4). Interestingly, one of the six SNPs within *Qs2-1A*, IWB45352, had a more significant effect ($p = 9.9 \times 10^{-6}$) on LMA than the originally reported IWB35476/*QLMA.wsu.1A* ($p = 0.0002$) (Figure S4A,B). The *Qs20-3B* locus (IWB65401, LOD 8.4) was located within 135 Mbp and 9 cM of *QLMA.wsu.3B* at IWB63008 (C. Liu, Parveen, et al., 2021; McNeil et al., 2009). *QLMA.wsu.3B* was significantly associated with LMA phenotype ($p = 0.02$), but not with the SI PHS trait ($p = 0.37$). *Qs20-3B*/IWB65401 showed a significant association with the PHS SI trait ($p = 4.2 \times 10^{-5}$) and a nearly significant association with LMA ($p = 0.054$). Examination of other highly significant PHS QTL revealed that *Qs31-5A*/IWB12023, *Qs39-5D*/IWB6788, and *Qs42-6A*/IWB30050 not only showed

a strong association with SI, but also with LMA phenotype ($p = 3.8 \times 10^{-6}$, 1.2×10^{-5}, and 0.03, respectively) (Figure S4E,F). Not all strong PHS QTL were associated with LMA phenotype. For example, neither *Qs35-5B* nor *Qs53-7D* showed a significant association with LMA (Figure S4G,H).

3.4 | Comparison of LMA, PHS, and vivipary phenotypes

Next, we examined whether LMA, PHS, and vivipary were correlated within the TCAP population. There was a significant correlation between LMA and PHS tolerance using BLUPs calculated for α-amylase activity over two greenhouse LMA induction experiments (from C. Liu, Parveen, et al. [2021]) and BLUPs of the summary statistic SI calculated over 2019 and 2020 (Figure 4a; $r = 0.52$, p-value = 0.009, $n = 226$). It may be difficult to see an association between PHS and LMA using Pearson's correlation given that LMA data are highly variable and weakly correlated between experiments (Butler et al., 2009; C. Liu, Parveen, et al., 2021). To address this, lines were placed into categories based on the number of times out of eight that LMA was induced (as in C. Liu, Parveen, et al. [2021]). Higher SI appeared to be more associated with the LMA susceptible (S) than the LMA resistant (R) category (Figure 4b).

To compare the three traits, vivipary germination assays were performed on a subset of 37 TCAP lines chosen to represent a range of LMA phenotypes. For vivipary assays, immature grains were hand-threshed at the soft dough stage of grain maturation (24–26 dpa, Zadok 84–85), plated on MS-agar, and their germination was scored daily. Incubation at the cooler temperature (18°C day/7.5°C night) resulted in significantly more germination than the warmer temperature (25°C day/18°C night) (p-value = 6.25×10^{-18}). Vivipary phenotypic classes included, full resistance resulting in low germination regardless of temperature (Figure 5a), moderate susceptibility resulting in higher germination at the cooler than the warmer incubation temperature (Figure 5b), and strong susceptibility resulting in high germination rates at both temperatures (Figure 5c).

Next, we examined whether there were any correlations between vivipary, LMA, and PHS phenotypes from spike-wetting tests using BLUPs for LMA. For vivipary, BLUPs of GI were calculated over the two experiments ($n = 37$). LMA BLUPs showed a moderate correlation to vivipary BLUPs from both the cool ($r = 0.61$, p-value = 6.4×10^{-5}) and the warm ($r = 0.64$, p-value 1.9×10^{-5}) incubation temperatures (Figure 6a,b). Sprout susceptibility was better correlated with vivipary at the cool ($r = 0.66$, p-value = 4.6×10^{-6}) than at the warm incubation temperature ($r = 0.58$, p-value = 1.0×10^{-4}) (Figure 6c,d). When the 37 TCAP lines characterized for vivipary were placed into phenotypic categories based on vivipary, PHS, and LMA, there were clear cases where the

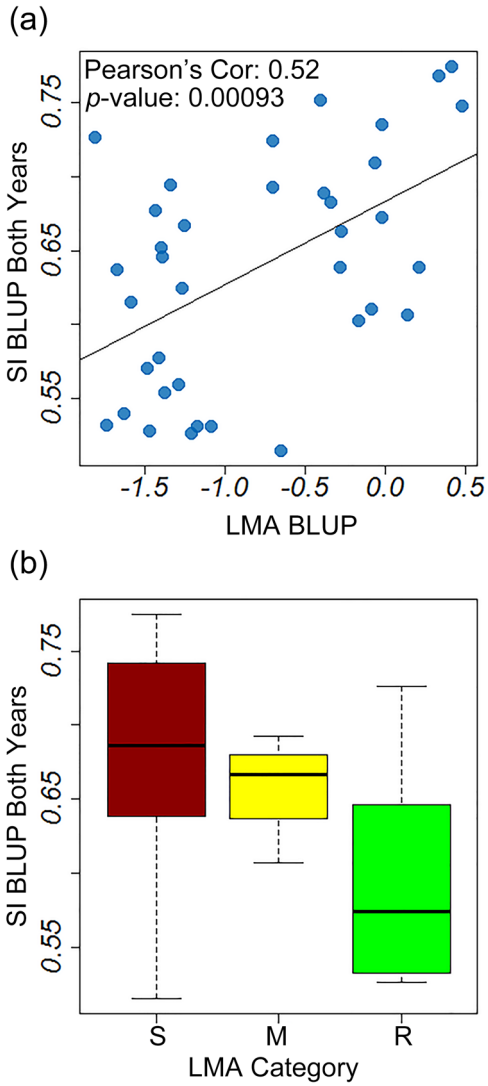

FIGURE 4 Correlations between PHS and LMA traits mapped in the TCAP. (a) A Pearson's correlation between BLUPs calculated for 2019 and 2020 PHS SI and BLUPs calculated for the 2018 and 2019 greenhouse LMA inductions (C. Liu, Parveen, et al., 2021) where enzyme activity was measured in absorbance units. (b) The effect of LMA phenotypic categories, LMA resistant (R), LMA moderate (M), and LMA susceptible (S), on sprouting index BLUPs. $N = 226$. BLUPs, best linear unbiased predictors; LMA, late maturity alpha-amylase; PHS, preharvest sprouting; TCAP, *Triticeae* coordinated agricultural project.

three phenotypes did not agree (Table S1). For example, CDC Utmost and WA8167 were PHS tolerant but susceptible to LMA and vivipary; AC Crystal and MN02072-7 were PHS susceptible but resistant to LMA and vivipary; and UI Pettit was susceptible to vivipary but resistant to PHS and LMA. This incomplete association of PHS, LMA, and vivipary suggests that some loci govern more than one of these traits. Of the QTL found to be associated with both PHS and LMA phenotype, *Qs2-1A*, *Qs39-5D*, and *Qs31-5A* were also significantly associated with vivipary in the subpopulation of 37 lines (Figure S4A,B). *Qs35-5B* is an example of a QTL that

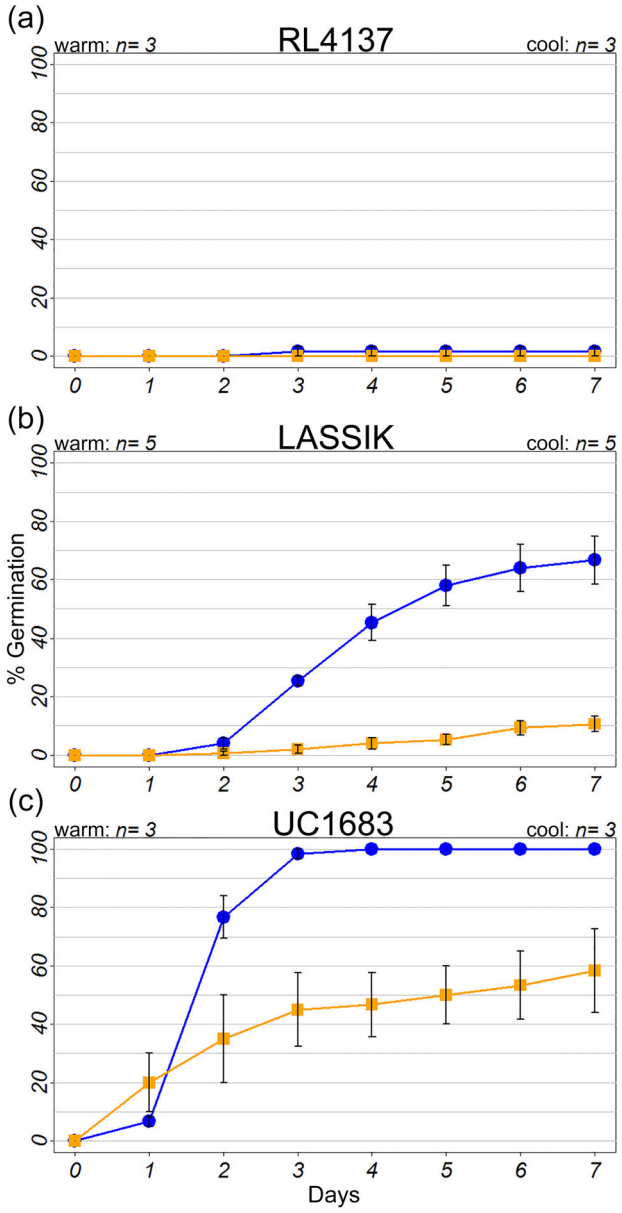

FIGURE 5 Examples of observed vivipary phenotypes. Immature grains (Zadok 85, soft dough stage) were plated on MS-agar and incubated under the warm 25˚C day/18˚C night (orange squares) or at the cool 18˚C day/7.5˚C night (blue circles) conditions. A vivipary-resistant line showed low germination at both temperatures (a), a moderately susceptible line germinated at the cooler temperature (b), and a highly susceptible line germinated well under both incubation temperatures (c). There were 30 grains per plate and three plates per treatment per genotype. MS, Murashige and Skoog.

was significantly associated with both PHS and vivipary, but not with LMA (Figure S4E).

3.5 | Discussion

The association study identified a high number of significant QTL using multiple and complementary approaches to evaluate the degree of sprouting and the time required to sprout. Analysis of an association panel representing ten North American spring wheat breeding programs identified 53 QTL for PHS tolerance, including six with a LOD score over 10 and 9 that were unique (Table S4). While 44 of these QTL colocalized with previously published loci, none of them colocalized within 10 cM of the cloned PHS tolerance genes *TaMFT-3A* or *TaMKK3-4A* (Nakamura et al., 2011; Shorinola et al., 2017). To the best of our knowledge, the only QTL within our study proximal to previously identified wheat genes was *Qs21-3D* located close to the *TaMFT-3A* homolog on chromosome 3D, but the functional significance of this homolog in PHS tolerance is unclear (Tillett et al., 2024). *Qs42-6A* mapped within 6.7 Mbp of a wheat homologue of the Arabidopsis ABF1 (ABA-Response Element Binding Factor1) gene involved in seed dormancy (Collin et al., 2021). A less significant QTL, *Qs45-6B*, mapped proximal to an ABF1 homolog on 6B. Future work will need to examine whether specific DNA polymorphisms in these genes are associated with the PHS phenotype.

The GWAS was performed using the sprouting score on each day of misting, Ts, and SI traits. There was little overlap between QTL detected based on sprouting scores on different days of misting, suggesting that they are distinct traits (Figure S2C). This may be because early sprouting scores are a better measure of germination capacity, whereas later sprouting scores measure rate of seedling growth. In contrast, there was greater overlap between loci detected using Ts at different sprouting score thresholds (Figure S2D). This may partly be due to the Ts values being derived from the same linear regression and partly due to reduced variability as a result of data smoothing over time (Figure 2a). Because this was the first study employing Ts, many sprouting score thresholds were used, possibly causing redundancy. In the current study, Ts1.1, 2.5, 3.5, and 5.5 detected most of the QTL. The 7 QTL detected solely at Ts7.5 were more likely a measure of post-germinative seedling growth than of germination per se.

While the use of multiple traits better represented the multidimensional data for GWAS, they also resulted in a large number of QTL detections necessitating subsequent analyses to focus on the most significant and reproducible QTL. Eleven key QTL were detected either repeatedly across years, with high LOD scores, and/or using all methods of representing the trait (Figure S3; Table 1). In particular, five QTL with strong effects and high LOD scores are good candidates for marker development: *Qs2-1A*, *Qs31-5A*, *Qs35-5B*, *Qs42-6A*, and *Qs53-7D* (Table 1; Table S4; Figures S3 and S4). Three QTL with a LOD over 10 were detected with all three types of traits, including *Qs2-1A*, *Qs35-5B*, and *Qs53-7D*. *Qs39-5D* (max LOD 8.7) was detected in all years using all types of traits. *Qs42-6A* was remarkable as the only QTL detected across all years using all three types of traits (sprouting score by day, SI, and Ts), and with multiple associations with a

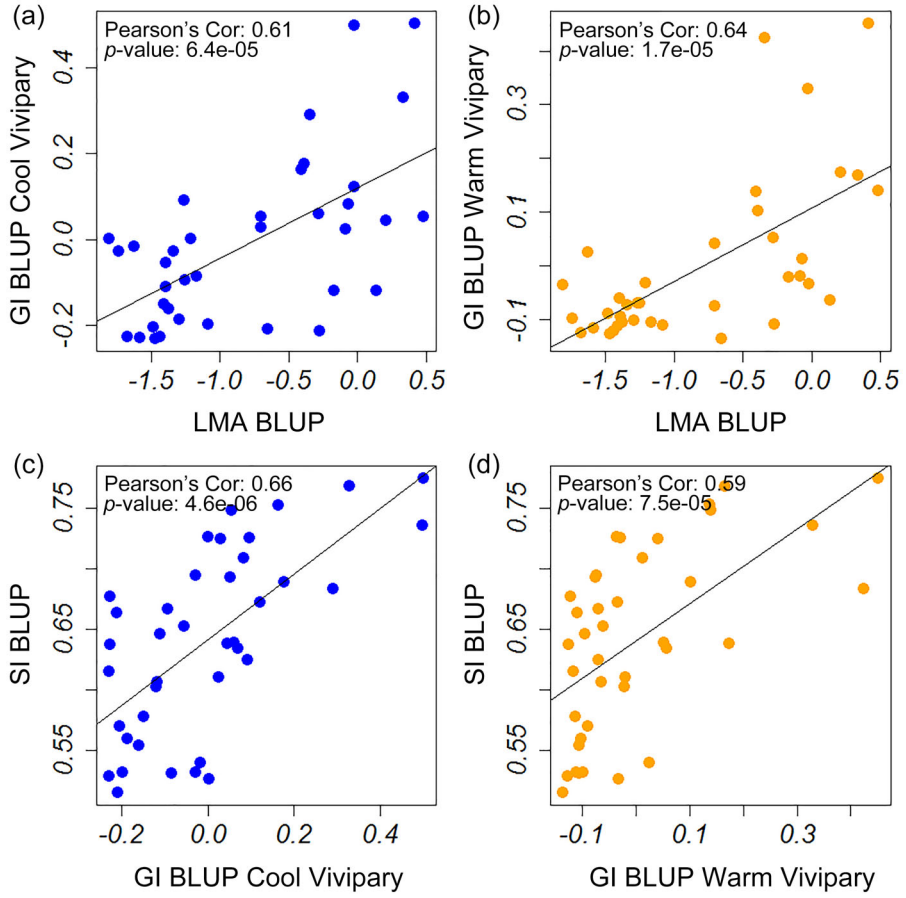

FIGURE 6 Comparisons of vivipary to late maturity alpha-amylase (LMA) and preharvest sprouting (PHS) phenotypes. Pearson's correlations between LMA best linear unbiased predictors (BLUPs) and germination index (GI) in vivipary plating assays incubated under (a) cool 18˚C day/7.5˚C night or (b) warm 25˚C day/18˚C night conditions. The correlations between PHS sprouting index (SI) and vivipary GI under (c) cool and (d) warm conditions. SI BLUPs were calculated over 2 years and GI BLUPs were calculated across two experiments. $N = 37$.

LOD over 10. Future work will need to validate QTLs in a biparental population, develop breeder-friendly markers, and identify the causative haplotype.

Additional good candidates for breeding include those QTL associated with tolerance not only to PHS, but also to LMA and vivipary. While our previous characterization of PHS, LMA, and vivipary suggested that they may be regulated by overlapping mechanisms, the eight lines examined were insufficient to generate correlations (Peery et al., 2023). The significant correlations between PHS, vivipary, and LMA in this study supported the hypothesis that they are genetically related (Figure 4; Figure 6). Moreover, favorable alleles of five QTL mapped for PHS tolerance also were significantly associated with higher LMA tolerance in the same panel: *Qs2-1A*, *Qs20-3B*, *Qs31-5A*, *Qs39-5D*, and *Qs42-6A* (Figure S4). Four major QTL mapped for PHS were also associated with vivipary tolerance, including *Qs2-1A*, *Qs31-5A*, *Qs39-5D*, and *Qs35-5B*. The overlap in PHS and LMA QTL is interesting because the LMA phenotype was elevated α-amylase, whereas the PHS and vivipary phenotype examined was visible germination. Alpha-amylase is induced during germination to mobilize stored starch reserves. Visible germination has been shown to correlate with increased α-amylase levels in some environments (Brown et al., 2018; DePauw & McCaig, 1991), but not always in environments with mild sprouting events (Martinez et al., 2018; Table S1). Because α-amylase is an integral part of germination, it is logical that it would be associated with visible sprouting. What is unclear is whether α-amylase expression can occur before the kernel is visibly sprouted. Future work will need to examine whether there is genetic variability in the timing of α-amylase induction during germination that may result in differences in cultivar susceptibility to low falling numbers in the field.

The connection between PHS, vivipary, and LMA will likely depend upon the specific alleles present in a cross or population. Selection for PHS tolerance, for example, will only result in LMA tolerance if one of the loci selected governs both phenomena. *Qs2-1A*, *Qs31-5A*, *Qs39-5D*, and *Qs42-6A* are good candidates for breeding because the same SNP was associated with both LMA and PHS tolerance (C. Liu et al., 2021; Table 1; Figure S4). Moreover, *Qs2-1A* colocalized with several previously mapped PHS QTL (Lin

et al., 2016; Lohwasser et al., 2013; Martinez et al., 2018; S. Wang et al., 2014). The favorable allele of *Qs20-3B* may have a weaker effect on LMA because it is only a neighboring locus to the LMA tolerance QTL identified in the TCAP and in Cranbrook/Halberd (C. Liu, Parveen, et al., 2021; Mrva & Mares, 2001). Future work will need to determine whether PHS and LMA tolerance in *Qs2-1A*, *Qs20-3B*, *Qs31-5A*, *Qs39-5D*, and *Qs42-6A* result from the same specific alleles or from neighboring loci. However, past breeding efforts may have benefited from the association between PHS and LMA tolerance at these loci, increasing LMA tolerance after selection only for PHS tolerance (C. Liu, Parveen, et al., 2021).

Cooler temperatures in vivipary experiments were significantly associated with higher GI (Figure 5; Peery et al., 2023), suggesting that the repression of germination during grain maturation depends in part on higher temperature. Dormancy loss in response to cold during grain maturation may be akin to dormancy loss in response to cold stratification of mature wheat grain (Tuttle et al., 2015; Xu et al., 2016). Cold stratification of mature grain is associated with increasing sensitivity to the germination-promoting hormone GA as well as decreased sensitivity to and accumulation of the dormancy-promoting hormone ABA. The expectation is that similar effects may be seen in LMA. Constitutive LMA without cool shock is associated with elevated ABA levels and with elevated levels of GA hormone precursors (Barrero et al., 2013). While no increase in bioactive GA was detected with cool induction of LMA, it is difficult to confidently conclude that there is no change because seed GA_1 levels are very low and difficult to measure accurately (Lenton & Appleford, 1991; D. Mares et al., 2022; D. Mares et al., 2024; Nelson et al., 2023). Further research should examine the effect of higher and lower temperatures on ABA and GA hormone levels during grain development. It is curious that moist chilling can break dormancy during maturation because cooler temperatures during grain maturation are known to increase dormancy and are associated with expression of the ABA-inducible gene *TaMFT-3A* (Nakamura et al., 2011). Moisture is likely the key determinant of whether the grain germinates prematurely or becomes quiescent and dormant at cool temperatures.

PHS, LMA, and vivipary differ not only in the timing, but in the localization of α-amylase expression (Mrva et al., 2006; Peery et al., 2023). The wheat endosperm is surrounded by a living aleurone cell layer that is the source of most of the α-amylase produced during germination, which in turn mobilizes starch as a nutrient for the germinating seedling (reviewed in Ritchie and Gilroy [1998]). During mature grain germination/PHS, GA hormone released by the embryo induces α-amylase expression first in the scutellum and the aleurone layer closest to the embryo, then later in the aleurone more distant from the embryo (Matthews et al., 2002). This leads to higher levels of α-amylase at the embryo-proximal than distal end of the grain. LMA, in contrast, causes the α-amylase to accumulate in random patches throughout the aleurone during grain maturation, leading to a more even distribution of α-amylase between the embryo proximal and distal ends (Mrva & Mares 1996; Mrva et al., 2006). When α-amylase levels were compared in half-grains of wheat, vivipary resulted in a pattern more similar to that of LMA than to PHS (Peery et al., 2023). LMA and vivipary also resemble each other in that α-amylase is expressed during grain maturation, before the grain becomes fully quiescent. Thus, LMA may be a partial activation of the germination program prior to the establishment of dormancy and may be more related to vivipary than mature grain germination. As discussed above, the prevailing hypothesis is that LMA results from cold temperature-triggered changes in ABA and GA signaling during grain development (reviewd by Kondare et al. [2015]; reviewed by Cannon et al. [2022]). Alternatively, LMA might also be a metabolic response to insufficient sugar from maternal or seed photosynthesis when a cold shock occurs during grain filling. Mobilization of starch by α-amylase may compensate for a sugar deficit caused by reduced photosynthetic rate in response to decreased or increased temperature (Hassan et al., 2021). From a survival standpoint, having more mature seed starch reserves may be less vital than having sufficient sugar to complete seed development. Future work should test whether LMA can be induced by conditions that reduce photosynthesis.

For breeding and molecular genetics, it can be preferable to have fewer strong QTL with strong effects than many minor QTL. The major loci emphasized in this study could be developed for marker-assisted selection or provide interesting targets for gene cloning or editing. The identification of many minor alleles suggests that genomic selection strategies may be able to take advantage of this variation to breed for PHS tolerance (Moore et al., 2017). Both the major and minor loci identified here could be employed as fixed effects in genomic prediction models or serve to validate findings in other mapping studies (Moore et al., 2017). Additionally, they may offer insights into which naturally occurring alleles are likely to be impactful in diverse germplasm and aid in efforts to combine sources of natural variation to breed for improved tolerance to PHS, LMA, and vivipary.

AUTHOR CONTRIBUTIONS

Scott W. Carle: Conceptualization; data curation; formal analysis; investigation; methodology; software; validation; visualization; writing—original draft; writing—review and editing. **Sarah R. Peery**: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; validation; visualization; writing—original draft; writing—review and editing. **Kimberly A. Garland-Campbell**: Conceptualization; methodology; project administration; resources; supervision; writing—review and

editing. **Michael O. Pumphrey**: Conceptualization; data curation; resources; writing—original draft. **Camille M. Steber**: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; supervision; writing—original draft; writing—review and editing.

ACKNOWLEDGMENTS

The authors thank R. Parveen, T. Harris, and V. Jitkov for expert technical assistance, and members of the Steber and Garland Campbell labs for helpful comments on the research and manuscript. Thanks are due to members of the Pumphrey, Steber, and Garland Campbell lab for assistance in the field. This work was funded by the USDA-ARS and the Washington Grain Commission.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The R script used to calculate Ts from sprouting scores is available as a public resource at: https://github.com/ScottCarleTheBiologist/Ts-Calculator.

ORCID

Scott W. Carle https://orcid.org/0000-0001-9885-5565
Kimberly A. Garland-Campbell https://orcid.org/0000-0003-4747-3270
Michael O. Pumphrey https://orcid.org/0000-0001-7524-9634
Camille M. Steber https://orcid.org/0000-0001-6255-7670

REFERENCES

Appels, R., Eversole, K., Stein, N., Feuillet, C., Keller, B., Rogers, J., Pozniak, C. J., Choulet, F., Distelfeld, A., & Poland, J., International Wheat Genome Sequencing Consortium (IWGSC). (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science*, *361*(6403), eaar7191.

Bajgain, P., Rouse, M. N., Bulli, P., Bhavani, S., Gordon, T., Wanyera, R., Njau, P. N., Legesse, W., Anderson, J. A., & Pumphrey, M. O. (2015). Association mapping of North American spring wheat breeding germplasm reveals loci conferring resistance to U44g99 and other African stem rust races. *BMC Plant Biology*, *15*(1), 1–19. https://doi.org/10.1186/s12870-015-0628-9

Barrero, J. M., Mrva, K., Talbot, M. J., White, R. G., Taylor, J., Gubler, F., & Mares, D. J. (2013). Genetic, hormonal, and physiological analysis of late maturity α-amylase in wheat. *Plant Physiology*, *161*(3), 1265–1277. https://doi.org/10.1104/pp.112.209502

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., Dai, B., Grothendieck, G., & Green, P. (2015). Package 'lme4'. *Convergence*, *12*(1), 2.

Brown, L. K., Wiersma, A. T., & Olson, E. L. (2018). Preharvest sprouting and α-amylase activity in soft winter wheat. *Journal of Cereal Science*, *79*, 311–318. https://doi.org/10.1016/j.jcs.2017.11.016

Butler, D. G., Tan, M. K., & Cullis, B. R. (2009). Improving the accuracy of selection for late maturity α-amylase in wheat using multi-phase designs. *Crop & Pasture Science*, *60*(12), 1202–1208. https://doi.org/10.1071/CP09124

Cannon, A. E., Marston, E. J., Kiszonas, A. M., Hauvermale, A. L., & See, D. R. (2022). Late-maturity α-amylase (LMA): Exploring the underlying mechanisms and end-use quality effects in wheat. *Planta*, *255*(1), Article 2. https://doi.org/10.1007/s00425-021-03749-3

Clark, D. R., & Monsanto Technology LLC. (2015). *Wheat cultivar WB6341*. U.S. Patent No. US8969688B2.

Collin, A., Daszkowska-Golec, A., & Szarejko, I. (2021). Updates on the role of ABSCISIC ACID INSENSITIVE 5 (ABI5) and ABSCISIC ACID-RESPONSIVE ELEMENT BINDING FACTORs (ABFs) in ABA signaling in different developmental stages in plants. *Cells*, *10*(8), 1996. https://doi.org/10.3390/cells10081996

Danquah, A., de Zélicourt, A., Boudsocq, M., Neubauer, J., Frei dit Frey, N., Leonhardt, N., Pateyron, S., Gwinner, F., Tamby, J. P., Ortiz-Masia, D., & Marcote, M. J. (2015). Identification and characterization of an ABA-activated MAP kinase cascade in *Arabidopsis thaliana*. *The Plant Journal*, *82*(2), 232–244. https://doi.org/10.1111/tpj.12808

DePauw, R. M., & McCaig, T. N. (1991). Components of variation, heritabilities and correlations for indices of sprouting tolerance and seed dormancy in *Triticum* spp. *Euphytica*, *52*, 221–229. https://doi.org/10.1007/BF00029399

Derera, N. F. (1989). *Preharvest field sprouting in cereals*. CRC Press.

Derkx, A., Baumann, U., Cheong, J., Mrva, K., Sharma, N., Pallotta, M., & Mares, D. (2021). A major locus on wheat chromosome 7B associated with late-maturity α-amylase encodes a putative ent-copalyl diphosphate synthase. *Frontiers in Plant Science*, *12*, Article 637685. https://doi.org/10.3389/fpls.2021.637685

Derkx, A. P., & Mares, D. J. (2020). Late-maturity α-amylase expression in wheat is influenced by genotype, temperature and stage of grain development. *Planta*, *251*(2), Article 51. https://doi.org/10.1007/s00425-020-03341-1

Finkelstein, R., Reeves, W., Ariizumi, T., & Steber, C. (2008). Molecular aspects of seed dormancy. *Annual Review Plant Biology*, *59*, 387–415. https://doi.org/10.1146/annurev.arplant.59.032607.092740

Godoy, J., Gizaw, S., Chao, S., Blake, N., Carter, A., Cuthbert, R., Dubcovsky, J., Hucl, P., Kephart, K., Pozniak, C., Prasad, P. V., Pumphrey, M. O., & Talbert, L. (2018). Genome-wide association study of agronomic traits in a spring-planted North American elite hard red spring wheat panel. *Crop Science*, *58*(5), 1838–1852. https://doi.org/10.2135/cropsci2017.07.0423

Graybosch, R. A., Baenziger, P. S., Santra, D., Regassa, T., Jin, Y., Kolmer, J., Bai, G., Amand, P. S., Chen, R., & Seabourn, B. (2019). Registration of 'Matterhorn' hard white waxy winter wheat. *Journal of Plant Registrations*, *13*(2), 207–211. https://doi.org/10.3198/jpr2018.09.0057crc

Hassan, M. A., Xiang, C., Farooq, M., Muhammad, N., Yan, Z., Hui, X., Yuanyuan, K., Bruno, A. K., Lele, Z., & Jincai, L. (2021). Cold stress in wheat: Plant acclimation responses and management strategies. *Frontiers in Plant Science*, *12*, Article 676884. https://doi.org/10.3389/fpls.2021.676884

Henderson, C. R. (1975). Best linear unbiased estimation and prediction under a selection model. *Biometrics*, *31*(2), 423–447. https://doi.org/10.2307/2529430

Heyne, E. G. (1959). Registration of improved wheat varieties, XXIII. *Agronomy Journal*, *51*, 689–692. https://doi.org/10.2134/agronj1959.00021962005100110019x

Himi, E., Mares, D. J., Yanagisawa, A., & Noda, K. (2002). Effect of grain colour gene (*R*) on grain dormancy and sensitivity of the embryo to abscisic acid (ABA) in wheat. *Journal of Experimental Botany*, *53*(374), 1569–1574. https://doi.org/10.1093/jxb/erf005

Himi, E., & Noda, K. (2005). Red grain colour gene (*R*) of wheat is a *Myb*-type transcription factor. *Euphytica*, *143*(3), 239–242. https://doi.org/10.1007/s10681-005-7854-4

Huang, M., Liu, X., Zhou, Y., Summers, R. M., & Zhang, Z. (2019). BLINK: A package for the next level of genome-wide association studies with both individuals and markers in the millions. *GigaScience*, *8*(2), giy154. https://doi.org/10.1093/gigascience/giy154

Ichinose, Y., Kuwabara, T., & Hakoyama, S. (2002). Germination of wheat grains at various temperatures in relation to the activities of α-amylase and endoprotease. *Plant Production Science*, *5*(2), 110–116. https://doi.org/10.1626/pps.5.110

King, R. W., & Richards, R. A. (1984). Water uptake in relation to preharvest sprouting damage in wheat: Ear characteristics. *Australian Journal of Agricultural Research*, *35*(3), 327–336. https://doi.org/10.1071/AR9840327

King, R. W., & von Wettstein-Knowles, P. (2000). Epicuticular waxes and regulation of ear wetting and pre-harvest sprouting in barley and wheat. *Euphytica*, *112*(2), 157–166. https://doi.org/10.1023/A:1003832031695

Kondhare, K. R., Farrell, A. D., Kettlewell, P. S., Hedden, P., & Monaghan, J. M. (2015). Pre-maturity α-amylase in wheat: The role of abscisic acid and gibberellins. *Journal of Cereal Science*, *63*, 95–108. https://doi.org/10.1016/j.jcs.2015.03.004

Kulwal, P., Ishikawa, G., Benscher, D., Feng, Z., Yu, L. X., Jadhav, A., Mehetre, S., & Sorrells, M. E. (2012). Association mapping for pre-harvest sprouting resistance in white winter wheat. *Theoretical and Applied Genetics*, *125*, 793–805. https://doi.org/10.1007/s00122-012-1872-0

Kulwal, P. L., Singh, R., Balyan, H. S., & Gupta, P. K. (2004). Genetic basis of pre-harvest sprouting tolerance using single-locus and two-locus QTL analyses in bread wheat. *Functional & Integrative Genomics*, *4*, 94–101.

Kumar, A., Kumar, J., Singh, R., Garg, T., Chhuneja, P., Balyan, H. S., & Gupta, P. K. (2009). QTL analysis for grain colour and pre-harvest sprouting in bread wheat. *Plant Science*, *177*(2), 114–122. https://doi.org/10.1016/j.plantsci.2009.04.004

Kumar, S., Knox, R. E., Clarke, F. R., Pozniak, C. J., DePauw, R. M., Cuthbert, R. D., & Fox, S. (2015). Maximizing the identification of QTL for pre-harvest sprouting resistance using seed dormancy measures in a white-grained hexaploid wheat population. *Euphytica*, *205*(1), 287–309. https://doi.org/10.1007/s10681-015-1460-x

Lenton, J. R., & Appleford, N. E. J. (1991). Gibberellin production and action during germination of wheat. In N. Takahashi, B. O. Phinney, & J. MacMillan (Eds.), *Gibberellins* (pp. 125–135). Springer. https://doi.org/10.1007/978-1-4612-3002-1

Lin, M., Cai, S., Wang, S., Liu, S., Zhang, G., & Bai, G. (2015). Genotyping-by-sequencing (GBS) identified SNP tightly linked to QTL for pre-harvest sprouting resistance. *Theoretical and Applied Genetics*, *128*(7), 1385–1395. https://doi.org/10.1007/s00122-015-2513-1

Lin, M., Zhang, D., Liu, S., Zhang, G., Yu, J., Fritz, A. K., & Bai, G. (2016). Genome-wide association analysis on pre-harvest sprouting resistance and grain color in US winter wheat. *BMC Genomics*, *17*, Article 794. https://doi.org/10.1186/s12864-016-3148-6

Liu, C., Parveen, R. S., Revolinski, S. R., Garland Campbell, K. A., Pumphrey, M. O., & Steber, C. M. (2021). The genetics of late maturity alpha-amylase (LMA) in North American spring wheat (*Triticum aestivum* L.). *Seed Science Research*, *31*(3), 159–168. https://doi.org/10.1017/S0960258521000064

Liu, C., Tuttle, K. M., Garland Campbell, K. A., Pumphrey, M. O., & Steber, C. M. (2021). Investigating conditions that induce late maturity alpha-amylase (LMA) using Northwestern US spring wheat (*Triticum aestivum* L.). *Seed Science Research*, *31*(3), 169–177. https://doi.org/10.1017/S0960258521000052

Liu, S., Sehgal, S. K., Li, J., Lin, M., Trick, H. N., Yu, J., Gill, B. S., & Bai, G. (2013). Cloning and characterization of a critical regulator for preharvest sprouting in wheat. *Genetics*, *195*, 263–273. https://doi.org/10.1534/genetics.113.152330

Liu, X., Huang, M., Fan, B., Buckler, E. S., & Zhang, Z. (2016). Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. *PLoS Genetics*, *12*, e1005767. https://doi.org/10.1371/journal.pgen.1005767

Lohwasser, U., Arif, R., & Börner, A. (2013). Discovery of loci determining pre-harvest sprouting and dormancy in wheat and barley applying segregation and association mapping. *Biologia Plantarum*, *57*(4), 663–674. https://doi.org/10.1007/s10535-013-0332-2

Maccaferri, M., Zhang, J., Bulli, P., Abate, Z., Chao, S., Cantu, D., Bossolini, E., Chen, X., Pumphrey, M., & Dubcovsky, J. (2015). A genome-wide association study of resistance to stripe rust (*Puccinia striiformis f. sp. tritici*) in a worldwide collection of hexaploid spring wheat (*Triticum aestivum* L.). *G3: Genes, Genomes, Genetics*, *5*(3), 449–465. https://doi.org/10.1534/g3.114.014563

Mao, X., Zhang, J., Liu, W., Yan, S., Liu, Q., Fu, H., Zhao, J., Huang, W., Dong, J., Zhang, S., & Yang, T. (2019). The *MKKK62-MKK3-MAPK7/14* module negatively regulates seed dormancy in rice. *Rice*, *12*(1), Article 2. https://doi.org/10.1186/s12284-018-0260-z

Mares, D., Derkx, A., Cheong, J., Zaharia, I., Asenstorfer, R., & Mrva, K. (2022). Gibberellins in developing wheat grains and their relationship to late maturity α-amylase (LMA). *Planta*, *255*(6), Article 119. https://doi.org/10.1007/s00425-022-03899-y

Mares, D., Derkx, A., & Mrva, K. (2024). Late maturity α-amylase (LMA) in gibberellin-insensitive, semi-dwarf wheat (*Triticum aestivum* L.). *Journal of Cereal Science*, *116*, 103858. https://doi.org/10.1016/j.jcs.2024.103858

Mares, D. J., & Mrva, K. (2001). Mapping quantitative trait loci associated with variation in grain dormancy in Australian wheat. *Australian Journal of Agricultural Research*, *52*(12), 1257–1265. https://doi.org/10.1071/AR01049

Mares, D. J., & Mrva, K. (2014). Wheat grain preharvest sprouting and late maturity alpha-amylase. *Planta*, *240*(6), 1167–1178. https://doi.org/10.1007/s00425-014-2172-5

Martinez, S. A., Godoy, J., Huang, M., Zhang, Z., Carter, A. H., Garland Campbell, K. A., & Steber, C. M. (2018). Genome-wide association mapping for tolerance to preharvest sprouting and low falling numbers in wheat. *Frontiers in Plant Science*, *9*, Article 141. https://doi.org/10.3389/fpls.2018.00141

Martinez, S. A., Tuttle, K. M., Takebayashi, Y., Seo, M., Garland Campbell, K. A., & Steber, C. M. (2016). The wheat ABA hypersensitive ERA8 mutant is associated with increased preharvest sprouting

tolerance and altered hormone accumulation. *Euphytica*, *212*(2), 229–245. https://doi.org/10.1007/s10681-016-1763-6

Matthews, P. R., Thornton, S., Gubler, F., White, R., & Jacobsen, J. V. (2002). Use of the green fluorescent protein to locate α-amylase gene expression in barley grains. *Functional Plant Biology*, *29*(9), 1037–1043. https://doi.org/10.1071/FP02011

McCarty, D. R. (1995). Genetic control and integration of maturation and germination pathways in seed development. *Annual Review of Plant Biology*, *46*(1), 71–93. https://doi.org/10.1146/annurev.pp.46.060195.000443

McMaster, G. J., & Derera, N. F. (1976). Methodology and sample preparation when screening for sprouting damage in cereals. *Cereal Research Communications*, *4*, 251–254.

McNeil, M. D., Diepeveen, D., Wilson, R., Barclay, I., McLean, R., Chalhoub, B., & Appels, R. (2009). Haplotype analyses in wheat for complex traits: Tracking the chromosome 3B and 7B regions associated with late maturity alpha amylase (LMA) in breeding programs. *Crop & Pasture Science*, *60*(5), 463–471. https://doi.org/10.1071/CP08340

Moore, J. K., Manmathan, H. K., Anderson, V. A., Poland, J. A., Morris, C. F., & Haley, S. D. (2017). Improving genomic prediction for pre-harvest sprouting tolerance in wheat by weighting large-effect quantitative trait loci. *Crop Science*, *57*(3), 1315–1324. https://doi.org/10.2135/cropsci2016.06.0453

Mrva, K., & Mares, D. J. (1996). Control of late maturity a-amylase synthesis compared to enzyme synthesis during germination. In K. Noda & D. J. Mares (Eds.), *Proceedings of the seventh international symposium on pre-harvest sprouting in cereals* (pp. 419–426). Center for Academic Societies.

Mrva, K., & Mares, D. J. (2001). Quantitative trait locus analysis of late maturity α-amylase in wheat using the doubled haploid population Cranbrook Halberd. *Australia Journal of Agricultural Research*, *52*, 1267–1273. https://doi.org/10.1071/AR01047

Mrva, K., Wallwork, M., & Mares, D. J. (2006). α-Amylase and programmed cell death in aleurone of ripening wheat grains. *Journal of Experimental Botany*, *57*(4), 877–885. https://doi.org/10.1093/jxb/erj072

Munkvold, J. D., Tanaka, J., Benscher, D., & Sorrells, M. E. (2009). Mapping quantitative trait loci for preharvest sprouting resistance in white wheat. *Theoretical and Applied Genetics*, *119*(7), 1223–1235. https://doi.org/10.1007/s00122-009-1123-1

Nakamura, S., Abe, F., Kawahigashi, H., Nakazono, K., Tagiri, A., Matsumoto, T., Utsugi, S., Ogawa, T., Handa, H., Ishida, H., & Mori, M. (2011). A wheat homolog of *MOTHER OF FT AND TFL1* acts in the regulation of germination. *The Plant Cell*, *23*(9), 3215–3229. https://doi.org/10.1105/tpc.111.088492

Narayanan, S., & Vara Prasad, P. V. (2014). Characterization of a spring wheat association mapping panel for root traits. *Agronomy Journal*, *106*(5), 1593–1604. https://doi.org/10.2134/agronj14.0015

Nelson, S. K., Kanno, Y., Seo, M., & Steber, C. M. (2023). Seed dormancy loss from dry after-ripening is associated with increasing gibberellin hormone levels in *Arabidopsis thaliana*. *Frontiers in Plant Science*, *14*, 1145414. https://doi.org/10.3389/fpls.2023.1145414

Paterson, A. H., Sorrells, M. E., & Obendorf, R. L. (1989). Methods of evaluation for preharvest sprouting resistance in wheat breeding programs. *Canadian Journal of Plant Science*, *69*(3), 681–689. https://doi.org/10.4141/cjps89-084

Peery, S. R., Carle, S. W., Wysock, M., Pumphrey, M. O., & Steber, C. M. (2023). LMA or vivipary? Wheat grain can germinate precociously during grain maturation under the cool conditions used to induce late maturity alpha-amylase (LMA). *Frontiers in Plant Science*, *14*, 1156784.

Prather, S., Schneider, T., Gaham Godoy, J., Odubiyi, S., Bosque-Perez, N. A., Rashed, A., Rynearson, S., & Pumphrey, M. O. (2022). Reliable DNA markers for a previously unidentified, yet broadly deployed Hessian fly resistance gene on chromosome 6B in Pacific Northwest spring wheat varieties. *Frontiers in Plant Science*, *13*, Article 779096. https://doi.org/10.3389/fpls.2022.779096

R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Ritchie, S., & Gilroy, S. (1998). Gibberellins: Regulating genes and germination. *The New Phytologist*, *140*(3), 363–383. https://doi.org/10.1111/j.1469-8137.1998.00299.x

Rodriguez, M. V., Barrero, J. M., Corbineau, F., Gubler, F., & Benech-Arnold, R. L. (2015). Dormancy in cereals (not too much, not so little): About the mechanisms behind this trait. *Seed Science Research*, *25*(2), 99–119. https://doi.org/10.1017/S0960258515000021

Ross, A. S., & Bettge, A. D. (2009). Passing the test on wheat end-use quality. In B. F. Carver (Ed.), *Wheat: Science and trade* (pp. 455–494). US Department of Agriculture. https://doi.org/10.1002/9780813818832.ch20

RStudio Team. (2019). *RStudio: Integrated development for R*. RStudio, Inc. http://www.rstudio.com/

Sato, K., Yamane, M., Yamaji, N., Kanamori, H., Tagiri, A., Schwerdt, J. G., Fincher, G. B., Matsumoto, T., Takeda, K., & Komatsuda, T. (2016). Alanine aminotransferase controls seed dormancy in barley. *Nature Communications*, *7*(1), Article 11625. https://doi.org/10.1038/ncomms11625

Schillinger, W. F., Papendick, R. I., Guy, S. O., Rasmussen, P. E., & Van Kessel, C. (2006). Dryland cropping in the western United States. *Dryland Agriculture*, *23*, 365–393.

Schramm, E. C., Abellera, J. C., Strader, L. C., Garland Campbell, K. A., & Steber, C. M. (2010). Isolation of ABA-responsive mutants in allohexaploid bread wheat (*Triticum aestivum* L.): Drawing connections to grain dormancy, preharvest sprouting, and drought tolerance. *Plant science*, *179*(6), 620–629. https://doi.org/10.1016/j.plantsci.2010.06.004

Shorinola, O., Balcárková, B., Hyles, J., Tibbits, J. F., Hayden, M. J., Holušova, K., Valárik, M., Distelfeld, A., Torada, A., Barrero, J. M., & Uauy, C. (2017). Haplotype analysis of the pre-harvest sprouting resistance locus *Phs-A1* reveals a causal role of *TaMKK3-A* in global germplasm. *Frontiers in Plant Science*, *8*, Article 1555. https://doi.org/10.3389/fpls.2017.01555

Sjoberg, S. M., Carter, A. H., Steber, C. M., & Garland-Campbell, K. A. (2020). Unraveling complex traits in wheat: Approaches for analyzing genotype × environment interactions in a multienvironment study of falling numbers. *Crop Science*, *60*(6), 3013–3026. https://doi.org/10.1002/csc2.20133

Somyong, S., Ishikawa, G., Munkvold, J. D., Tanaka, J., Benscher, D., Cho, Y. G., & Sorrells, M. E. (2014). Fine mapping of a preharvest sprouting QTL interval on chromosome 2B in white wheat. *Theoretical and Applied Genetics*, *127*, 1843–1855. https://doi.org/10.1007/s00122-014-2345-4

Tan, M. K. (2004). Molecular marker development for LMA determinant in 'Spica' and 'Cranbrook'. *VAWCRC Report*, *42*(18), 1–17.

Tillett, B. J., Vetch, J. M., Martin, J. M., & Giroux, M. J. (2024). Novel alleles of *MFT-A* and *MFT-B1* appear to impact wheat preharvest sprouting in *Triticum aestivum* and *Triticum turgidum* ssp. *durum*. *Cereal Chemistry*, *101*(5), 1160–1168. https://doi.org/10.1002/cche.10789

Torada, A., Koike, M., Ogawa, T., Takenouchi, Y., Tadamura, K., Wu, J., Matsumoto, T., Kawaura, K., & Ogihara, Y. (2016). A causal gene for seed dormancy on wheat chromosome 4A encodes a MAP kinase kinase. *Current Biology*, *26*, 782–787. https://doi.org/10.1016/j.cub.2016.01.063

Tuttle, K. M., Martinez, S. A., Schramm, E. C., Takebayashi, Y., Seo, M., & Steber, C. M. (2015). Grain dormancy loss is associated with changes in ABA and GA sensitivity and hormone accumulation in bread wheat, *Triticum aestivum* (L.). *Seed Science Research*, *25*(2), 179–193. https://doi.org/10.1017/S0960258515000057

Walker-Simmons, M. (1987). ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. *Plant Physiology*, *84*(1), 61–66. https://doi.org/10.1104/pp.84.1.61

Wang, J., & Zhang, Z. (2021). GAPIT version 3: Boosting power and accuracy for genomic association and prediction. *Genomics, Proteomics & Bioinformatics*, *19*(4), 629–640. https://doi.org/10.1016/j.gpb.2021.08.005

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., Lillemo, M., Mather, D., … Akhunov, E., International Wheat Genome Sequencing Consortium. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*, 787–796. https://doi.org/10.1111/pbi.12183

Warner, R. L., Kudrna, D. A., Spaeth, S. C., & Jones, S. S. (2000). Dormancy in white-grain mutants of Chinese Spring wheat (*Triticum aestivum* L.). *Seed Science Research*, *10*, 51–60. https://doi.org/10.1017/S0960258500000064

Xi, W., Liu, C., Hou, X., & Yu, H. (2010). *MOTHER OF FT AND TFL1* regulates seed germination through a negative feedback loop modulating ABA signaling in *Arabidopsis*. *The Plant Cell*, *22*(6), 1733–1748. https://doi.org/10.1105/tpc.109.073072

Xu, Q., Truong, T. T., Barrero, J. M., Jacobsen, J. V., Hocart, C. H., & Gubler, F. (2016). A role for jasmonates in the release of dormancy by cold stratification in wheat. *Journal of Experimental Botany*, *67*(11), 3497–3508. https://doi.org/10.1093/jxb/erw172

Zadocks, J. C., Chang, T. T., & Konzak, C. F. (1974). The growth stage code for cereals. *Weed Research*, *14*, 415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zanetti, S., Winzeler, M., Keller, M., Keller, B., & Messmer, M. (2000). Genetic analysis of pre-harvest sprouting resistance in a wheat × spelt cross. *Crop Science*, *40*(5), 1406–1406. https://doi.org/10.2135/cropsci2000.4051406x

Zuo, J., Lin, C. T., Cao, H., Chen, F., Liu, Y., & Liu, J. (2019). Genome-wide association study and quantitative trait loci mapping of seed dormancy in common wheat (*Triticum aestivum* L.). *Planta*, *250*, 187–198. https://doi.org/10.1007/s00425-019-03164-9

SUPPORTING INFORMATION

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

How to cite this article: Carle, S. W., Peery, S. R., Garland-Campbell, K. A., Pumphrey, M. O., & Steber, C. M. (2025). Association mapping of preharvest sprouting tolerance in spring wheat reveals genetic connections to late maturity alpha-amylase and vivipary. *Crop Science*, *65*, e70169. https://doi.org/10.1002/csc2.70169