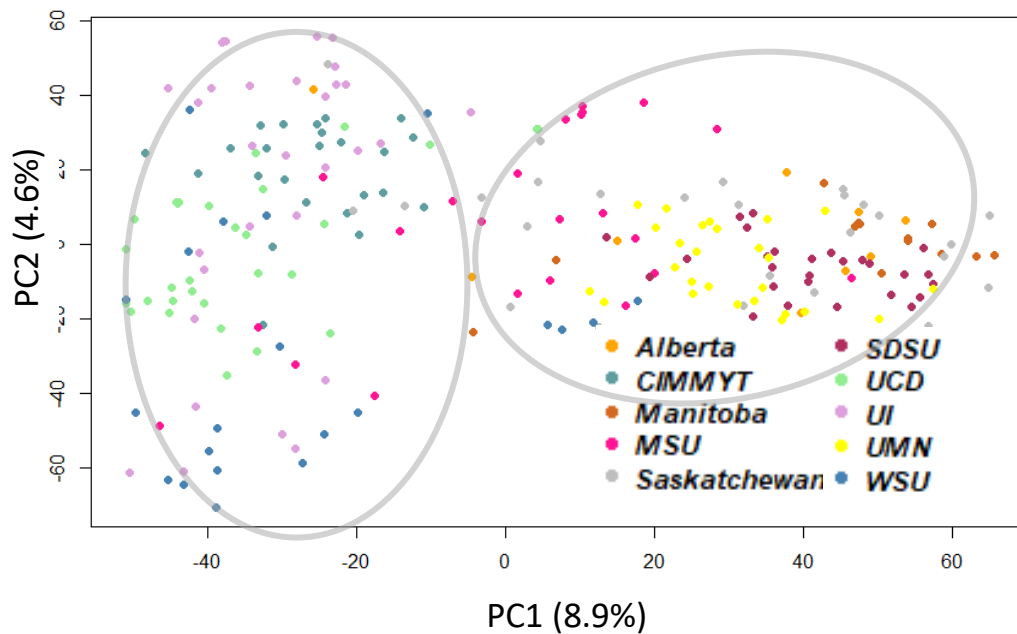


The Genetics of LMA in North American  
Spring Wheat (*Triticum aestivum* L.)

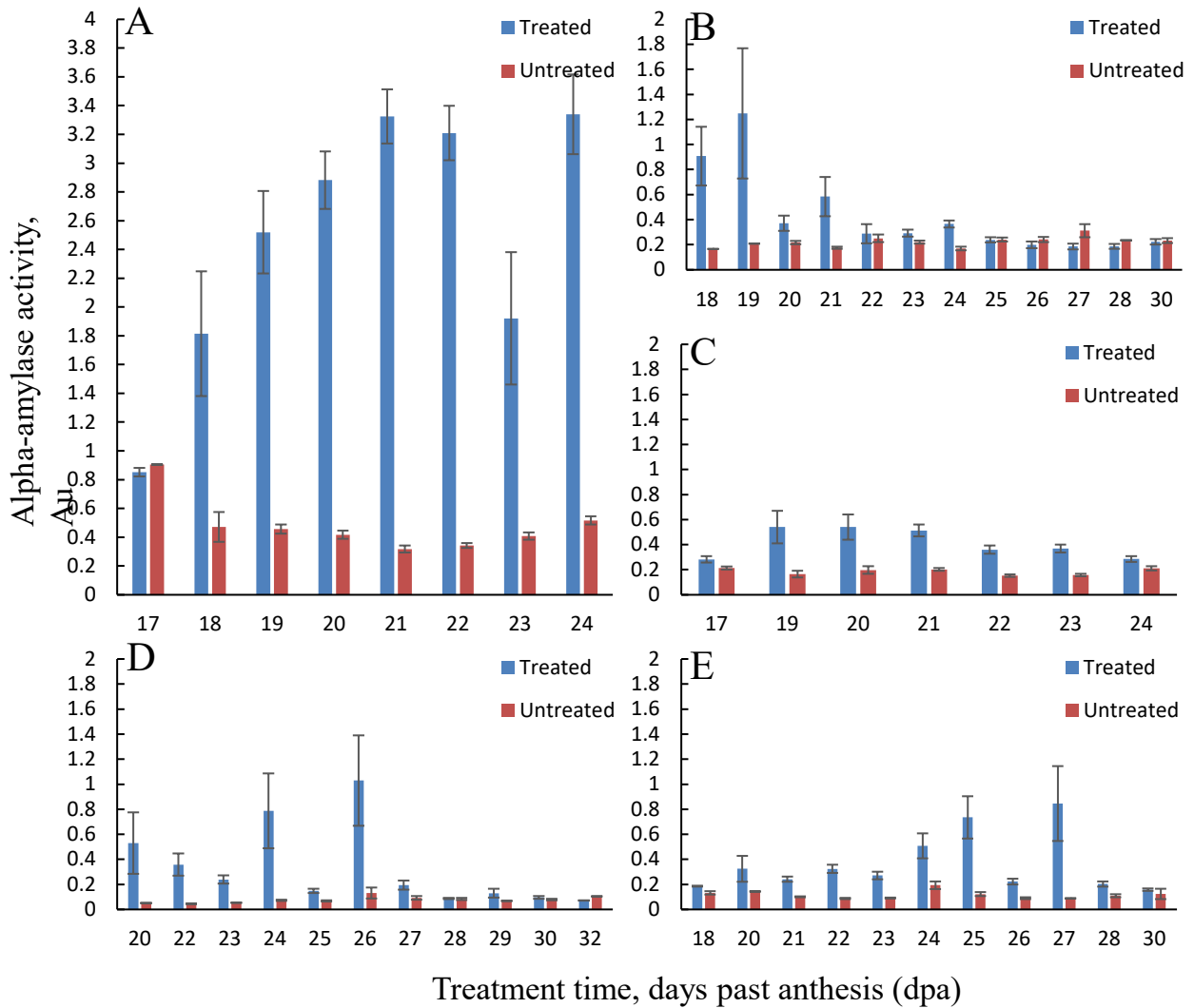
Chang (Chloe) Liu<sup>1</sup>, Rehana S. Parveen<sup>1</sup>,  
Samuel R. Revolinski<sup>1</sup>, Kimberly A. Garland  
Campbell<sup>1,2</sup>, Michael O. Pumphrey<sup>1\*</sup>,  
Camille M. Steber<sup>1,2\*</sup>

Affiliations: <sup>1</sup>Department of Crop and Soil  
Science, Washington State University,  
Pullman WA, and <sup>2</sup>USDA-ARS, Wheat  
Health, Genetics and Quality Unit, Pullman  
WA 99164-6420.

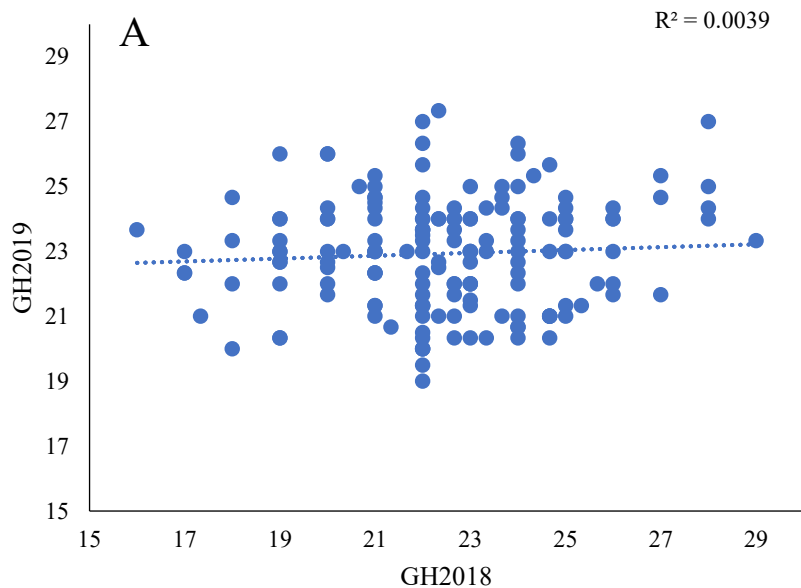
Supplemental Figures S1, S2, S3, S4, and  
S5.



**Fig. S1.** Principal component analysis of TCAP population based on 19,192 SNPs. PC1 explains 8.9% genetic variation, and PC2 explains 4.6% genetic variation. The mapping population was comprised on hard spring varieties representing 10 North American breeding programs that fell into two principal components: the first representing Canada and the upper Midwestern U.S.; the second representing the western U.S. and CIMMYT in Mexico.

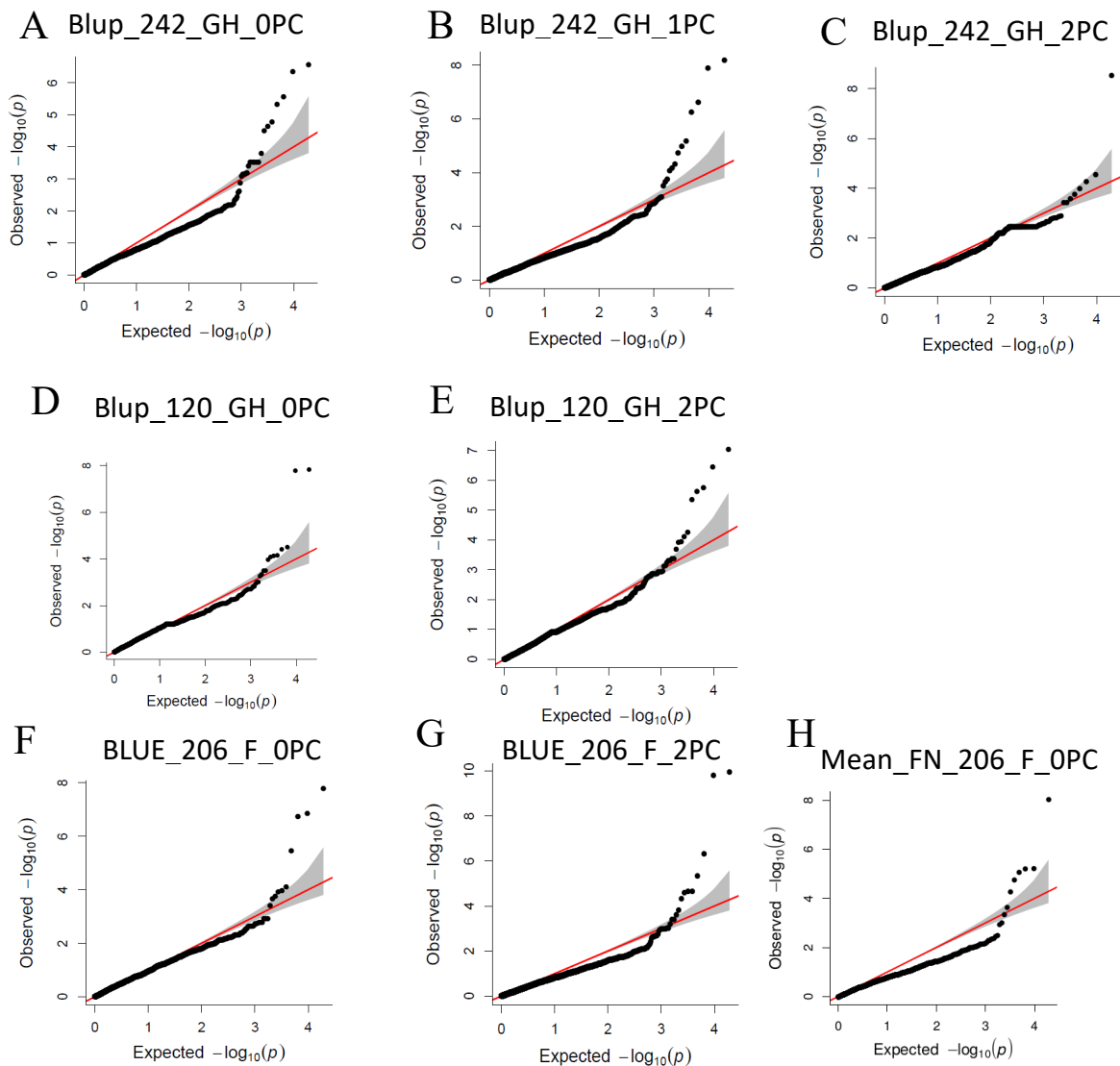


**Fig. S2.** A preliminary LMA time course experiment was performed to determine if some TCAP varieties induce LMA earlier than 26 dpa. Treated plants were placed into a cool chamber with an 18°C day/ 7.5°C night for 7 days beginning at the indicated number of days past anthesis. Time course experiments were performed on cultivars: A) UC1599, B) Glencross, C) Lillian, D) GP069, and E) Scarlet. Glencross and Lillian induce LMA earlier than UC1599 or Scarlet. Note that UC1599 expressed  $\alpha$ -amylase at elevated levels (0.4 Au) without cold treatment but shows an additional 10-fold induction with cold treatment. Thus, UC1599 was designated as an “LMA constitutive”. For each LMA time point, treated and untreated  $\alpha$ -amylase levels were measured for between 3 and 16 spikes.

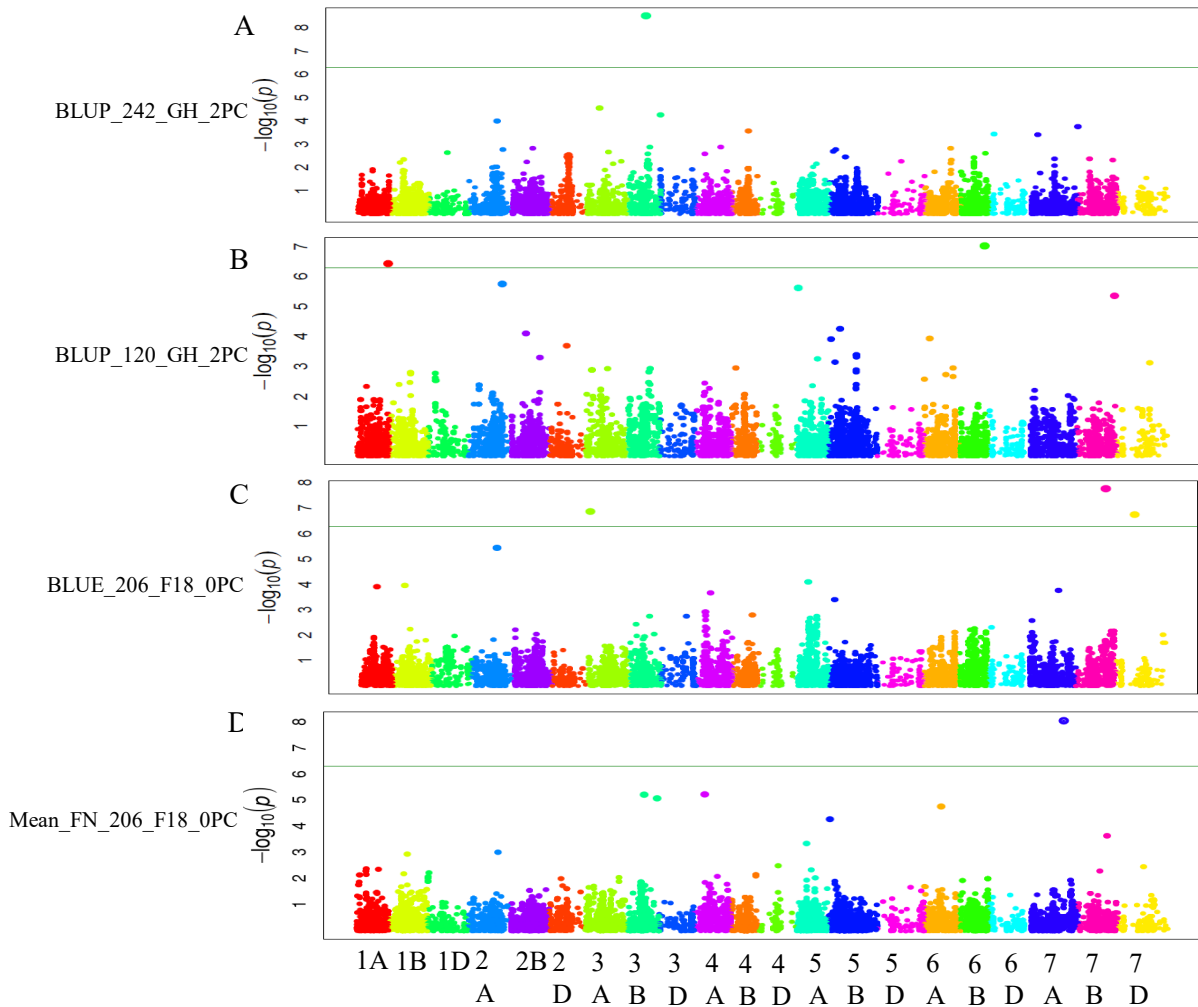


<b>B</b>	Expt	Run	Lines	Anthesis range
	GH2018	1	1-125	Nov 16– Dec 4, 2017
	GH2018	2	126-190	Aug 6-16, 2018
	GH2018	3	191-256	Oct 5-20, 2018
	GH2019	4	1-125	Mar 9-25, 2019
	GH2019	5	126-256	Apr 26 – May 9, 2019

**Fig. S3.** Comparison of LMA treatment time (dpa) and the timing of anthesis in GH2018 and GH2019. A) There is no correlation between LMA treatment time in days past anthesis in GH2018 and GH2019 experiments. B) Comparison of anthesis timing for each run in the GH2018 and GH2019 experiments. The three runs used to test all lines in GH2018 covered a wider range of dates than the two runs in GH2019.

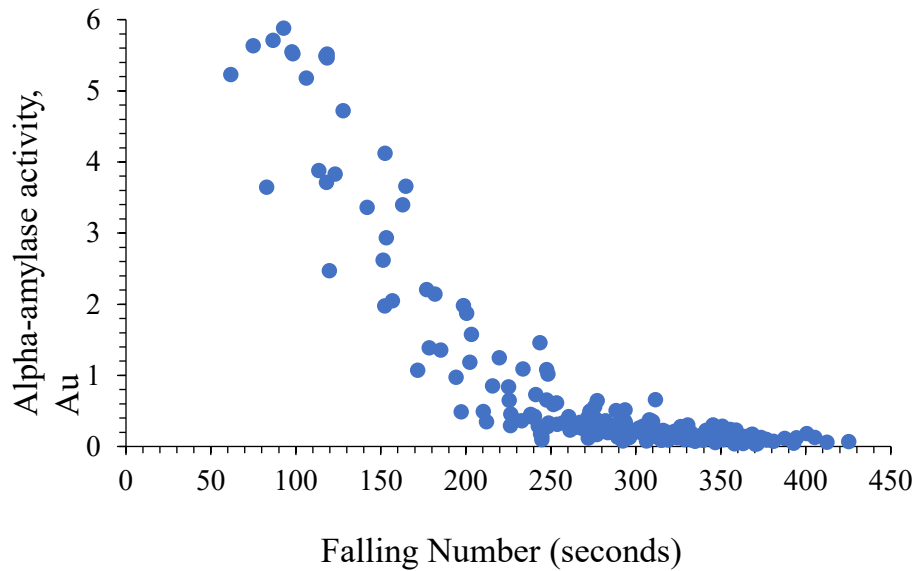


**Fig. S4.** QQ-plots of each GWAS using the greenhouse (GH) and the field (F) data. The ideal QQ plot has datapoints along a 45 degree angle (red line) until the end where significant loci deviate from the red line. If the datapoints fall below the red line, then the model is over-compensating for the population structure, if they fall above then there is insufficient compensation in the model. A-C) A better plot was obtained using two principal components (PC) than using zero or one PC based on BLUPs of the 242 lines in GH2018 and GH2019 experiments. D-E) A better fit was obtained using two PCs than no principal components based on the BLUPs of 120 lines representing the 25% tails of GH2018 and GH2019. No significant QTL were mapped with one PC. F-G) A better fit was obtained using no principal components than two PCs based on BLUEs of the 206 lines examined in the F2018 experiment. No significant QTL were mapped with one PC. (H) Using FN from F2018, a single significant QTL was mapped using 0 PC. The average of 2 field replicates was used.



**Fig. S5.** Manhattan plots showing of QTL mapped using FarmCPU.

Significant marker-trait associations appear as data points above the green line representing the negative  $\log_{10}$  of the Bonferroni adjusted  $p$ -value of 0.05 divided by the number of markers used for association mapping. A) *QLMA.wsu.3B* was mapped to chromosome 3B using BLUPs of the 242 lines in GH2018 and GH2019 using 2PCs. B) *QLMA.wsu.1A* and *QLMA.wsu.6B* were mapped using the BLUPs of 25% tails (120 lines) in GH2018 and GH2019 using 2PCs. C) *QLMA.wsu.3A*, *QLMA.wsu.7B*, and *QLMA.wsu.7D* were mapped using the BLUEs of F2018 (206 lines) in F2018 using no PCs. D) No significant QTL were identified using falling number data from F2018 (206 lines) using the mean of two biological replications and no principal components.



**Fig. S6. Comparison of alpha-amylase enzyme activity and falling number in F2018.** The alpha-amylase enzyme activity (Au) and falling number (sec) from cold-treated samples in the first biological replicate of the TCAP population grown in the Field in 2018.