Minnesota Wheat Research and Promotion Council
FULL RESEARCH PROPOSAL TEMPLATE
For Crop Year 2026 (01/01/2026 to 12/31/2026)

Project Title: Characterization of late maturity α-amylase (LMA) susceptibility in hard red spring wheat

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Project Period: 01/01/26 – 12/31/26
Estimated cost: $ 10,700

Abstract - Low Hagberg Falling Number (HFN) events continue to cause substantial economic losses in spring wheat and lead to steeper market discounts for producers. Preharvest sprouting (PHS) is the main reason for elevated α-amylase levels and remains the primary cause of low HFN. However, recent years have shown low FN in otherwise PHS-resistant varieties and during seasons without rainy conditions before harvest. This suggests the involvement of another phenomenon, known as late maturity α-amylase (LMA), which is a stress-induced expression of α-amylase triggered by temperature shock during late grain filling, particularly in the absence of post-maturity rainfall. While PHS is well characterized and one of the important goals for breeding programs, little to no research has been done on the LMA problem in hard red spring (HRS) wheat. The goal of this project is to evaluate key HRS wheat genotypes for LMA susceptibility using a validated controlled-environment assay and enzymatic quantification. As a secondary objective, we will explore the usability of near-infrared (NIR) for screening α-amylase/FN as an alternative to enzyme activity assays. This research will help assess variation in LMA expression and improve an in-house LMA testing pipeline for future studies.

Background and Importance - Late-maturity α-amylase (LMA) is a developmental misexpression of α-amylases caused by cool temperatures (or heat shocks in some cases) during the late grain maturation period. Unlike PHS, LMA results in more widespread α-amylase expression throughout the aleurone layer and often does not cause visible sprout symptoms. It has also been suggested that the low HFN associated with LMA does not impact end-use quality as strongly as high α-amylase caused by PHS. Unfortunately, the current HFN method cannot distinguish between these underlying phenomena, and LMA-driven low HFN also triggers unwarranted price discounts for farmers. In our multilocation trials from recent years, we observed multiple incidents of low FN in otherwise PHS-resistant varieties and during seasons without rainy conditions before harvest. However, these locations experienced low temperatures (mid-40s F) during the grain development period, indicating a role for LMA. If the latter is true, LMA could continue affecting producers until there is an approach to differentiate between the two causes of HFN.

LMA susceptibility has been a significant concern in Australia and the Pacific Northwest (PNW) region of the US. This trait has been previously characterized in both Australia and the PNW, where significant variation in LMA susceptibility or tolerance has been observed, which can be utilized for breeding purposes. However, little is

known about the degree of LMA susceptibility or tolerance in HRS germplasm. To address this, we propose a pilot study to investigate the prevalence of LMA susceptibility in a set of HRS wheat varieties and advanced lines, focusing specifically on those showing low HFN despite a good PHS rating. Additionally, previous studies have indicated that LMA expression is highly sensitive to genotype-by-environment interactions, emphasizing the importance of locally calibrated protocols. The information generated from this work can be utilized in future research to inform breeding decisions, identify genotypes for genetic characterization, and improve an in-house LMA testing pipeline.

Research Methods - We will phenotype late maturity α-amylase (LMA) susceptibility in hard red spring (HRS) wheat using a developmentally timed, cold-shock induction in controlled environments, followed by α-amylase enzyme assays. The appropriate stage (~ Zadoks 85) to induce LMA using cold shock will be determined by calculating days-to-anthesis (DTA) and visual inspection of developing grain to target the soft-dough window, where LMA induction is most reproducible. Each genotype will be evaluated in two sets: one as a control and the other subjected to cold shock treatment, followed by an enzymatic activity assay for both sets. Enzyme activity will be quantified with a Phadebas 96-well assay using plate standards and corrections.

Experiment 1: The objective of experiment 1 is to characterize LMA susceptibility in 12-15 HRS wheat genotypes using a validated controlled-environment assay. We will conduct three independent experiments for the entire study, each with two to three technical replicates. Within each independent set, two pots will be grown for a given genotype in the greenhouse (22-24°C day/15-17°C night). The first three tillers of each pot will be tagged when the florets in the center of the spike reach anthesis. At the appropriate stage (based on DTA and visual inspection), one pot will be moved to the growth chamber for cold shock treatment (18°C day/7.5°C night) with a 16 h photoperiod and <65% relative humidity. After the 7-day cold treatment, the pots will be returned to the warm chamber and allowed to mature at 25°C during the day/18°C at night for 3–4 weeks. Three treated and three untreated spikes that reached anthesis within a 3-d range will be hand-harvested and threshed. The three spikes from a pot will be treated as three samples for statistical analysis.

Experiment 2: The objective of Experiment 2 is to measure the enzymatic activity for treated and untreated samples from Experiment 1.

Experiment 3: Before the laboratory analysis of α-amylase, the reflectance of each sample will be measured with near-infrared spectroscopy. Subsequently, a preliminary statistical model will be evaluated to estimate the ability of NIR to predict the α-amylase concentration.

Timeline:

Year	*Period*	*Activity*
2026	Spring	Perform the LMA induction experiment in controlled environment
	Summer	Experiment 2 and Experiment 3
	Fall	Experiment 2 and Experiment 3, Data Analysis for project summary

Dissemination of Results: This pilot project will fill a critical gap by generating the first controlled assessment of LMA susceptibility in HRS germplasm. The resulting dataset will be shared as a report with breeders to inform breeding decisions and lay the groundwork for future genetic mapping. If appropriate, the results could be published as a peer-reviewed article and/or presented at a relevant meeting. If successful, we plan to use the techniques developed in this pilot study to conduct a more thorough investigation of LMA cold shock induction of all lines in the UMN statewide HRS Variety Trial (about 75 entries) in 2027.

List potential collaborators or co-investigators you may consider inviting to participate: No others at this time
Budget requirements: $10,700. Personnel: 5% salary and fringe of postdoc Harsimar Gill ($4,350); undergraduate labor to assist Drs. Gill and Annor ($5,000) for growth and care of plants and enzymatic and NIR assays; Supplies for alpha-amylase assay ($600); Greenhouse and growth chamber rental ($750)

Submit full proposal (max. 2 pages) and itemized budget to bsorenson@mnwheat.com by 11:59 PM, 11/15/25