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Extended grain fill stabilizes malt barley quality across variable environments and nitrogen inputs

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Abstract

Barley (*Hordeum vulgare* L.) intended for malting must meet quality standards for producers to receive premium prices. In semiarid climates, variable weather can reduce grain quality and lead to over- or underfertilization, further compromising economic returns. The stay-green trait may support producers' attainment of malt quality standards across variable environments and nitrogen (N) inputs by sustaining photosynthesis during grain filling through earlier heading and or delayed senescence. We identified three barley cultivars carrying distinct combinations of stay-green-associated quantitative trait loci (QTLs), with Buzz and MT Endurance exhibiting extended grain fill relative to the cultivar Hockett. This study evaluated whether variation in N response among the cultivars could be attributed to their stay-green-associated QTL combinations and resulting effects on malt quality. Cultivars were grown under four N treatments across four locations from 2019 to 2023. Extended grain fill in Buzz and MT Endurance improved the attainability of producer market traits, particularly in trials with elevated temperatures, reduced precipitation, and overfertilization. Under these conditions, Hockett exceeded grain protein thresholds and failed to meet kernel plumpness standards, whereas the extended grain fill

Abbreviations: AA, alpha-amylase; AMBA, American Malting Barley Association; BG, beta-glucan; DP, diastatic power; FAN, free amino nitrogen; KASP, kompetitive allele-specific PCR; MSU, Montana State University; QTL, quantitative trait locus; RCBD, randomized complete block design; SNP, single nucleotide polymorphism; TWT, test weight.

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cultivars maintained acceptable protein concentrations and higher plumpness. These responses were associated with stay-green-associated QTLs, which shift the balance toward starch accumulation during grain filling and help maintain malt quality under variable N and environmental conditions. These findings provide a basis for further investigation into how stay-green traits and their associated QTLs enhance the starch-to-protein ratio, enabling barley to maintain key producer market traits under elevated N and environmental stress.

Plain Language Summary

Farmers often grow barley to sell as malt, a key ingredient in beer, because it earns a higher price when quality standards are met. However, weather and nitrogen management can affect grain quality, as too much nitrogen under stressful conditions can increase protein and reduce seed size, causing farmers to lose the premium. This research focused on three genetic regions controlling the stay-green trait, which lengthens the grain development period, allowing seeds to fill with protein, starch, and other compounds important for malt quality. Barley cultivars with differing gene versions in the stay-green regions were compared across Montana locations under varying nitrogen levels. Cultivars with gene versions that extended grain development the longest had lower protein and plumper grains under stressful weather and high nitrogen conditions. Stay-green barley can help farmers maintain malt quality under challenging conditions, improving crop stability and profitability.

1 | INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cereal crop in the northern and intermountain regions of the United States, as the multifaceted grain is used for human consumption, livestock feed, and the production of beer and spirits. Montana, North Dakota, and Idaho are the leading barley-producing states, and malt barley provides producers greater economic value than feed barley, with prices averaging 48% higher from 2010 to 2023 (USDA NASS, 2025a, 2025b). However, producers in semiarid regions often struggle to meet stringent malt quality standards, resulting in grain being downgraded to feed and reducing profitability.

Malt quality is defined by numerous grain and malt criteria established by the American Malting Barley Association (AMBA), including barley and malt factors encompassing measures of malt modification, wort composition, and enzyme activity (AMBA, 2025). However, grain protein concentration and kernel plumpness are primary determinants of market acceptance and producer premiums, as these traits govern whether a barley crop qualifies for malting markets. AMBA thresholds specify $\leq 13\%$ protein and $>90\%$ plump kernels (6/64-in. sieve), underscoring the importance of these traits for economic return (AMBA, 2025).

Achieving consistent malt quality remains challenging due to interactions among climate variability and nitrogen (N)

management. Late-season drought and heat stress shorten grain filling, reducing kernel plumpness while increasing grain protein concentration, frequently resulting in grain that fails to meet malting specifications (Bohačenko et al., 2021; Cai et al., 2013; Mahalingam & Bregitzer, 2018). In semiarid barley producing regions, precipitation variability and increasing temperatures are expected to limit water availability during grain fill (Knapp et al., 2023; NOAA NCEI, 2021; Whitlock et al., 2017). Although Montana guidelines recommend 1.2 pounds of available N per bushel of expected yield for malt barley (McVay et al., 2017), environmental uncertainty complicates fertility management, as preseason N decisions rely on expected yield under variable growing conditions. Consequently, mismatches between N availability and environmental conditions frequently result in protein and plumpness levels that fail to meet malting standards.

Genetic traits that extend grain fill duration and enhance stress resilience may provide a strategy for stabilizing malt quality under semiarid conditions and variable N fertility. The stay-green trait is characterized by prolonged grain fill duration, resulting from earlier heading and/or delayed senescence, which allows the plant to sustain photosynthetic activity for an extended period during kernel development (Kamal et al., 2019; Thomas & Ougham, 2014; Williams et al., 2022). Stay-green genotypes have been associated with improved yield stability and quality under drought stress (Gous et al., 2013;

Williams et al., 2022). Quantitative trait loci (QTLs) associated with stay-green phenotypes have also been linked to terminal heat stress resistance, suggesting a shared physiological basis for stress adaptation (Gous et al., 2015). Post-anthesis assimilates derived from continued photosynthesis contribute to starch accumulation and kernel size, while N remobilization from pre-anthesis reserves largely influences grain protein concentration (Barmeier et al., 2021). These physiological relationships suggest stay-green genotypes may help maintain kernel plumpness while moderating grain protein concentration under environmental stress.

Recent studies have identified QTLs associated with grain fill duration that also influence grain quality traits relevant to malting. Williams et al. (2024) reported QTLs colocating with kernel plumpness and grain protein concentration, including *QGFhd-2H*, which was associated with heading date, grain fill duration, plumpness, and protein content. Similarly, *QGFmt-6H* was associated with maturity, grain fill duration, protein concentration, plumpness, and test weight (TWT) (Williams et al., 2024); this region also contains the *HVNAMI* NAC transcription factor, previously linked to senescence regulation (Alptekin et al., 2021; Distelfeld et al., 2008; See et al., 2002). The *QGFhd-7H* locus has also been associated with heading date, grain fill duration, and grain protein stability across environments (Alqudah et al., 2014; Dang et al., 2022; Williams et al., 2024). Collectively, these loci suggest that genetic control of grain fill duration may influence both yield stability and malt quality.

Despite evidence linking stay-green traits to stress tolerance and yield stability, their influence on malt quality across semiarid environments and variable N fertility remains poorly understood. Genetic variation affecting grain fill duration may influence grain protein concentration and kernel plumpness, yet the extent to which stay-green associated QTLs stabilize malt quality under variable environmental and fertility conditions remains unclear. Therefore, this study evaluated how stay-green-associated QTL combinations in elite malting germplasm from the Montana State University (MSU) Barley Breeding Program influence grain fill duration, agronomic performance, and malt quality across diverse environments and N fertility levels. We hypothesized that stay-green alleles prolong grain fill duration, enhance starch accumulation, and stabilize grain protein concentration and kernel plumpness, thereby improving malt quality under semiarid conditions and reducing economic risk for producers.

2 | MATERIALS AND METHODS

2.1 | DNA isolation, genotyping, and plant material selection

Genomic DNA was extracted from leaf tissue samples of Montana barley breeding material using a modified SDS-

Core Ideas

- Producers struggle to meet malt standards due to semiarid climates, causing economic loss.
- Stay-green quantitative trait loci (QTLs) improve grain quality and stability under environmental stress and N variability.
- Extended grain fill supports an optimal starch-to-protein ratio.

based protocol (Alptekin et al., 2021). DNA concentration was quantified using a NanoDrop ND-2000c spectrophotometer (Thermo Fisher Scientific). Kompetitive allele-specific PCR (KASP) markers targeting three stay-green-associated QTLs (*QGFhd-2H*, *QGFmt-6H*, and *QGFhd-7H*; Table S1) were developed from single nucleotide polymorphisms (SNPs) reported by Williams et al. (2024). KASP primers were designed using the KASP-on-Demand system (LGC, Biosearch Technologies), and genotyping followed the standard 61°C–55°C touchdown protocol.

Hockett (PVP-201000058; Bearpaw/ND7593), Buzz (PVP-202000161; ND7293/Bearpaw, Hockett ×4//LK644, Lewis/Karl), and MT Endurance (PVP-submitted; Hockett/ND24388) were selected for field evaluation based on contrasting grain fill duration and allelic variation at the targeted stay-green QTLs (Williams et al., 2024). Genome-wide SNP data were available for all cultivars from the Illumina BarleySNP50K array (Bayer et al., 2017) or the Illumina 9K iSelect Custom Genotyping BeadChip (Comadran et al., 2012), providing genomic context for phenotypic comparisons among cultivars.

2.2 | Experimental design and site descriptions

Between 2019 and 2023, we evaluated barley cultivars under four N rate treatments across seven field trials conducted at four locations in Montana, including two distinct locations in Bozeman, as well as sites in Huntley and Conrad (Table 1). These environments represent semiarid production conditions typical of malt barley systems in Montana. Buzz and Hockett were included in all trials, while MT Endurance was included beginning in 2021 (Table 1).

Dryland trials in the Bozeman region were conducted at the MSU Arthur H. Post Agronomy Farm (45.37° N, 111.14° W; 1456 m ASL) in 2019, 2020, and 2023, and at the Fort Ellis Research Farm (45.67° N, 110.97° W; 1505 m ASL) in 2022 (Table 1). Within each Bozeman area location-year, N treatments were applied to four fixed field sections due to field-scale application constraints. Nitrogen treatments were

TABLE 1 Overview of barley field trials evaluating barley cultivars under varying nitrogen treatments.

Location	Site	Year	Cultivars	Nitrogen		
				Treatment	Applied kg N ha ⁻¹	Recommended kg N ha ⁻¹ based on environment
Bozeman	Arthur H. Post Agronomy Farm	2019	Buzz and Hockett	0.5x	149	96
				1.0x	175	191
				1.5x	262	287
				2.0x	350	382
		2022	Buzz, MT Endurance, and Hockett	0.5x	56	40
				1.0x	117	80
				1.5x	164	120
				2.0x	217	160
	Fort Ellis Research Farm	2023	Buzz, MT Endurance, and Hockett	0.5x	99	98
				1.0x	166	195
				1.5x	232	293
				2.0x	305	390
		2020	Buzz and Hockett	0.5x	90	90
				1.0x	166	180
				1.5x	249	271
				2.0x	328	361
Huntley	Southern Agricultural Research Center	2020	Buzz and Hockett	0.5x	106	67
				1.0x	188	133
				1.5x	282	200
				2.0x	377	226
		2021	Buzz, MT Endurance, and Hockett	0.5x	101	69
				1.0x	202	139
				1.5x	303	208
				2.0x	404	277
Conrad	Western Triangle Agricultural Research Center	2021	Buzz, MT Endurance, and Hockett	0.5x	121	32
				1.0x	135	63
				1.5x	202	95
				2.0x	269	126

Note: Applied nitrogen (N) values are listed as the amount applied by trial managers based on rates necessary to achieve target yield goals based on historical averages specific to each location. Recommended N rates were calculated by first averaging the actual yield of all cultivars within the 1.0x N treatment for each location-year. The guideline of 1.2 lb N per expected bushel of yield was then applied to this average to determine the recommended N rate for the 1.0x treatment (McVay et al., 2017). This calculated rate was subsequently used to derive the recommended rates for the 0.5x, 1.5x, and 2.0x N treatments.

therefore not randomized or replicated at the field level. Within each N treatment section, cultivars were arranged in a randomized complete block design (RCBD) with three replicates. As a result, cultivar comparisons within each N treatment are statistically supported, whereas N treatment effects and cultivar × N responses at these locations are interpreted descriptively.

Fully replicated trials were conducted at the Southern Agricultural Research Center (45.92° N, 108.24° W; 919 m ASL) in Huntley, MT, in 2020 and 2021, and at the Western Triangle Agricultural Research Center (48.31° N, 111.93° W; 1132 m ASL) in Conrad, MT, in 2021 (Table 1). Huntley trials were irrigated using impact sprinklers to maintain approxi-

mately 50% available soil moisture through the soft dough stage, following regional best management practices outlined by McVay et al. (2017). Conrad trials were conducted under dryland conditions representative of one of the driest barley-producing regions in Montana. At both locations, experiments were arranged as a RCBD with three replicates, and cultivar and N rate treatments were factorially randomized within each block. N fertilizer was applied at the plot level, and border plots were maintained between adjacent plots to minimize cross-plot contamination.

Across all environments, N treatments consisted of 0.5x, 1.0x, 1.5x, and 2.0x levels, where the 1.0x treatment was intended to represent the recommended N rate of 1.2 lb N

TABLE 2 Weather conditions for each trial location and year.

Field trial condition	Bozeman					Huntley			Conrad	
	2019	2020	2022	2023	Historic mean	2020	2021	Historic mean	2021	Historic mean
Precipitation (mm)										
Preseason	151	210	140	196	164	184	121	134	142	113
April	84	22	42	19	44	20	30	39	31	30
May	44	20	110	19	67	40	49	61	83	52
June	52	93	60	129	69	121	10	57	22	59
July	68	17	14	25	33	27	3	28	19	27
August	19	14	15	47	32	18	38	26	48	32
Total	418	376	381	435	409	410	251	345	345	313
Mean maximum (°C)										
April	12	13	9	11	13	13	14	15	14	14
May	15	18	15	20	18	20	20	21	17	19
June	23	22	23	21	23	26	31	26	28	23
July	26	27	30	28	28	31	35	31	31	28
August	28	29	31	27	28	32	29	30	26	27

Note: Preseason precipitation was calculated as the total from September of the previous year through March of the trial year. Total precipitation includes September of the previous year through August of the trial year. Historic means are reported for the periods 1969–2024 for Bozeman, 1953–2024 for Huntley, and 1971–2024 for Conrad.

per expected bushel of barley (McVay et al., 2017) (Table 1). Residual soil N was determined through preplant soil testing (Table S2), and urea application rates were calculated to achieve target yield goals based on historical averages for each location. To assess whether applied N exceeded crop demand, the observed mean grain yield of all cultivars within the 1.0x N treatment for each location-year was multiplied by the guideline of 1.2 lb N per expected bushel of grain (McVay et al., 2017) to estimate an environment-specific recommended 1.0x N rate (Table 1). Overfertilization was considered to have occurred when the estimated recommended N rate was lower than the applied N rate. Recommended nitrogen rates for the 0.5x, 1.5x, and 2.0x treatments were subsequently derived from the environment-specific 1.0x rate (Table 1).

Monthly precipitation and temperature data for each growing season, along with historical averages, were obtained from NOAA climatological summaries (Table 2).

2.3 | Agronomic measurements

Heading and physiological maturity dates were recorded in the Bozeman trials to quantify grain fill duration. Heading was defined as the Julian date when 50% of plants within a plot reached head emergence (Zadok stage 59; Zadoks et al., 1974). Physiological maturity was recorded when 50% of spikes had senesced and kernels reached the hard dough stage (Zadok stage 89; Zadoks et al., 1974). Grain fill duration was calculated as the number of days between heading and physiological maturity. Grain fill duration was not determined at

other locations because heading and maturity dates were not recorded.

Across all trials, grain yield was determined from harvested grain weight and adjusted for plot area and grain moisture content. Grain protein and moisture content were measured using near-infrared spectroscopy (Infratec NOVA, FOSS). Kernel plumpness was measured as the percentage of kernels retained on a 6/64 × 3/4-in. sieve following 30 s of shaking on a Pfeuffer Sortimat (Pfeuffer GMBH). Grain TWT was measured using a John Grain Analysis Computer 2500-UGMA (Corn Belt Testing Inc.).

2.4 | Malt measurements

Microscale malting was conducted at the MSU Malt Quality Laboratory using steep/germination vessels and a kiln (Custom Laboratory Products). Malting procedures followed protocols described by Turner et al. (2019) and were adjusted among years to achieve a target steep-out moisture content of 45% and to optimize malt modification for each growing season (Table S4).

In 2019, malt extract was determined using fine-grind extract. In subsequent years, coarse-grind extract was used to better reflect brewhouse conditions. Malt quality was evaluated using the Congress mash procedure (American Society of Brewing Chemists) through 2022 with minor modifications described by Turner et al. (2019). In 2023, a single-infusion isothermal mash at 65°C replaced the Congress Mash protocol to better reflect current brewing practices.

To account for methodological differences among years, cultivars and N treatment effects on malt quality were evaluated within each location-year. The following malt quality parameters were measured following the procedures described by Turner et al. (2019): malt extract, diastatic power (DP), free amino nitrogen (FAN), ratio of soluble to total protein (S/T), beta-glucan (BG), and alpha-amylase (AA).

2.5 | Statistical analysis

Statistical analyses were conducted in R (R Core Team, 2024). Analyses were conducted separately for each location-year due to differences in experimental design among locations. Linear mixed-effects models were fitted using the “lme4” package (Bates et al., 2015) to evaluate the effects of N treatment, cultivar, and their interaction on agronomic and malt quality traits.

At Bozeman locations, N treatments were applied to fixed sections and were not randomized or replicated. Within each N treatment section, cultivars were arranged in a RCBD design with three blocks. Models therefore included N treatment, cultivar, and their interaction as fixed effects, with block nested within N treatment included as a random effect. Since N treatments were not replicated at these locations, inference for N main effects and cultivar $\times$ N interactions is descriptive, whereas cultivar effects are supported by replicated observations within N sections.

At Conrad and Huntley, experiments were conducted as RCBDs with three replicates and factorial combinations of cultivar and N treatment randomized within blocks. Models included cultivar, N treatment, and their interaction as fixed effects, with block included as a random effect. At these locations, inference of N main effects and cultivar $\times$ N interactions is statistical supported.

Analysis of variance was performed using the “car” package (Fox & Weisberg, 2019). Estimated marginal means and Tukey-adjusted pairwise comparisons were obtained using the “emmeans” package (Lenth & Piaskowski, 2023). At locations with replicated N treatments (Conrad and Huntley), Tukey-adjusted comparisons were performed across all cultivar $\times$ N combinations within each location-year. At Bozeman, where N treatments were not replicated, Tukey-adjusted comparisons were applied only to cultivar differences within each N treatment, and N main effects and cultivar $\times$ N responses are presented descriptively.

Pearson correlation coefficients were calculated within each location-year using plot-level data to evaluate relationships among grain fill duration, agronomic traits, and malt quality traits. Correlations were computed using the “Hmisc” package (Harrell, 2020). Relationships between grain fill duration and selected agronomic and malt quality traits were

visualized with scatterplots and fitted linear regression lines using “ggplot2” (Wickham, 2016).

3 | RESULTS

3.1 | Genetic characterization of barley cultivars

Genotyping identified allelic variation among cultivars at three stay-green-associated QTLs previously reported by Williams et al. (2024) (Table S1). Target loci were genotyped using KASP markers developed for this study (Table S3). Buzz carries stay-green alleles at *QGFmt-6H*, associated with delayed maturity, and *QGFhd-2H*, associated with earlier heading. MT Endurance carries stay-green alleles at *QGFmt-6H* (delayed maturity) and *QGFhd-7H* (earlier heading), whereas Hockett carries only the *QGFhd-7H* stay-green allele. Thus, cultivars differed in both the number and combination of stay-green-associated alleles related to grain fill duration.

Genome-wide genotyping indicated additional genetic differences beyond the targeted QTLs. Using the Illumina BarleySNP50K array, Hockett and Buzz differed at 5623 of 44,040 SNP loci (13%). Similarly, Hockett and MT Endurance differed at 1647 of 7842 loci (21%) using the 9K Illumina Infinium iSelect.

3.2 | Environmental parameters

Precipitation during the grain filling period of development (June–July) varied across location-years, resulting in contrasting levels of water availability during kernel development (Table 2). At the Bozeman locations, precipitation was below the long-term average in multiple years, particularly in June 2019 and 2022 and in July 2020, 2022, and 2023. Similarly, Conrad in 2021 experienced below-average precipitation in both June and July. In contrast, precipitation at Huntley was generally near or above the long-term average, and supplemental irrigation was applied as needed to minimize water stress.

Temperature patterns during grain filling also differed among environments (Table 2). At Bozeman, mean maximum temperatures in June were comparable to the long-term average, whereas July temperatures exceeded the long-term average in 2022 and 2023 (Table 2). At Huntley, mean maximum temperatures in June were above the long-term average in both 2020 and 2021, while July temperatures exceeded the long-term average in 2021 (Table 2). Similarly, mean maximum temperatures at Conrad in 2021 were above the long-term average in both June and July (Table 2). These

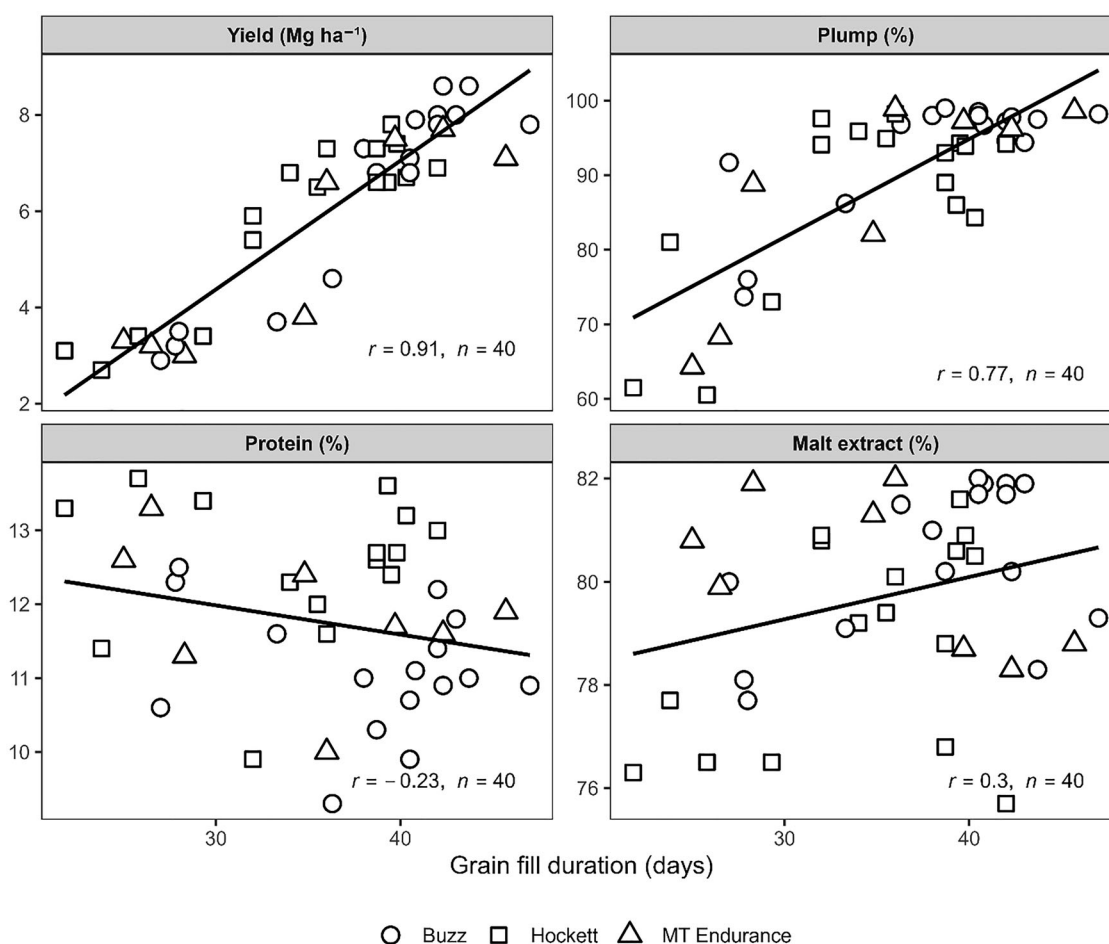


FIGURE 1 Relationships between grain fill duration and key agronomic and malt quality traits at the Bozeman locations. Panels show associations between grain fill duration and grain yield, kernel plumpness, grain protein concentration, and malt extract across cultivar $\times$ nitrogen treatment observations from 2019, 2020, 2022, and 2023. Symbols indicate cultivar. Solid lines represent linear regressions fitted across observations within each trait. Pearson correlation coefficients (r) and sample sizes (n) are shown in each panel.

conditions resulted in location-years with differing combinations of temperature and precipitation during grain fill.

3.3 | Relationship between grain fill duration, agronomic traits, and malt quality

Pearson correlations were used to evaluate relationships between grain fill duration and agronomic and malt quality traits at the Bozeman location using data pooled across N treatments within each year (Figure 1; Table S5). Across years, longer grain fill duration was generally associated with increased grain yield and kernel plumpness and reduced grain protein concentration (Figure 1; Table S5). TWT showed weak but generally positive correlations with grainfill duration (Table S5).

Grain fill duration was also associated with multiple malt quality traits relevant to malting performance (Figure 1; Table S5). Across years, longer grain fill duration was generally

associated with higher malt extract, S/T, FAN, and AA, and with lower DP and BG (Figure 1; Table S5). Although the strength of relationships varied among years, the overall direction of associations was consistent across environments (Table S5).

3.4 | Relationships between grain protein, plumpness, and malt quality traits

Pearson correlations were used to evaluate relationships among grain protein concentration, kernel plumpness, and malt quality traits across location-years using data pooled across N treatments (Figure S1; Table S5). Grain protein concentration and kernel plumpness were consistently negatively associated across environments, reflecting their opposing roles in determining malting suitability.

Grain protein concentration showed consistent negative associations with malt extract and S/T and positive

associations with DP across all location-years. Grain protein was also generally positively associated with BG, whereas relationships with AA and FAN varied among environments (Figure S1; Table S5).

Overall, greater kernel plumpness was associated with improved malt quality traits across environments. Higher plumpness was associated with increased malt extract and S/T and lower BG (Figure S1; Table S5). However, plumpness was negatively associated with DP across location-years. Associations between plumpness and AA and FAN were weaker and more variable among environments (Figure S1; Table S5).

3.5 | Cultivar agronomic performance across environments

Cultivar agronomic performance is presented with consideration of site-specific experimental design. At the Bozeman locations, N effects and cultivar $\times$ N response are interpreted descriptively, whereas at Conrad and Huntley, where N treatments were replicated, N and cultivar $\times$ N effects were statistically evaluated (Table S6).

Heading and maturity dates differed among cultivars at Bozeman in most years (Table S6). Buzz tended to head earlier and mature later than Hockett, while MT Endurance also headed earlier and matured slightly later than Hockett (Table S7). Differences between Buzz and MT Endurance were relatively small, although Buzz typically matured slightly later (Table S7). Consequently, Buzz and MT Endurance consistently exhibited longer grain fill duration than Hockett across environments and N treatments (Figure 2; Table S7). Grain fill duration generally increased with N rate, although responses were not strictly linear across location-years (Figure 2).

Differences in grain yield among cultivars were generally limited, although cultivar effects were detected in some environments. Yield differences were observed in Bozeman (2019 and 2023) and Huntley (2020) (Table S6), where cultivar ranking varied by N rate (Table S7). In Bozeman 2019, Buzz produced higher yield than Hockett at the 1.5x and 2.0x N rates, while in Bozeman 2023 Buzz yielded more than Hockett at the 1.5x N rate (Table S7). In contrast, at Huntley in 2020, Hockett yielded higher than Buzz at the 2.0x N rate (Table S7). Across environments, yield differences among cultivars were inconsistent, and no cultivar exhibited a consistent yield advantage across N treatments.

Grain protein concentration increased with N rate across environments (Figure 2; Table S7). Hockett exhibited the higher protein than Buzz and MT Endurance, particularly at the 1.5x and 2.0x N rates (Figure 2; Table S7). Buzz often had similar or lower protein than MT Endurance. Differences among cultivars were most evident at higher N rates, where Hockett showed a stronger increase in protein relative to Buzz and MT Endurance.

Kernel plumpness showed an inverse response to N relative to protein, decreasing as N rate increased (Figure 2; Table S7). Buzz and MT Endurance maintained greater plumpness across N rates, whereas Hockett exhibited larger reductions in plumpness at higher N levels (Figure 2; Table S7).

TWT varied among cultivars and environments (Table S6). MT Endurance exhibited slightly lower TWT than Buzz and Hockett in several environments, while differences between Buzz and Hockett were inconsistent across location-years (Figure 2; Table S7).

3.6 | Cultivar malt quality across environments

Malt extract varied among cultivars and N treatments (Table S8). Across environments, MT Endurance consistently exhibited the highest malt extract, followed by Buzz, while Hockett typically had the lowest extract values (Figure 3; Table S9). Differences among cultivars were most pronounced at the higher N rates (1.5x and 2.0x), where MT Endurance and Buzz typically maintained higher extract relative to Hockett (Figure 3; Table S9). In several environments, including Bozeman and Huntley in 2020, extract declined with increasing N in Hockett, whereas Buzz showed comparatively smaller reductions (Figure 3; Table S9).

BG differed among cultivars but showed less consistent response to N rate (Table S8). Across environments, Hockett typically exhibited the highest BG, Buzz the lowest, and MT Endurance intermediate values (Figure 3; Table S9). Although BG responses to N varied among location-years, cultivar ranking remained largely consistent (Table S9).

FAN increased with increasing N rate across environments (Figure 3; Table S9). Buzz and MT Endurance typically exhibited higher FAN than Hockett across N treatments, while differences between Buzz and MT Endurance were small (Table S9). In Bozeman 2022, MT Endurance exceeded Buzz at most N rates, although cultivar differences were otherwise consistent across environments (Table S9).

AA showed consistent cultivar differences with limited response to N rate (Table S8). Buzz exhibited higher AA than Hockett and MT Endurance across most location-years and N treatments (Figure 3; Table S9), while MT Endurance and Hockett were similar across environments.

DP varied across cultivars and N treatments and tended to increase with increasing N rate (Figure 3; Table S9). Hockett generally exhibited the highest DP, whereas Buzz and MT Endurance showed similar or lower DP across most environments (Figure 3; Table S9).

S/T differed among cultivars and N treatments (Table S8). Buzz and MT Endurance consistently exhibited higher S/T than Hockett across environments (Figure 3; Table S9). In several environments, including Bozeman and Huntley 2020, S/T declined with increasing N rate (Figure 3).

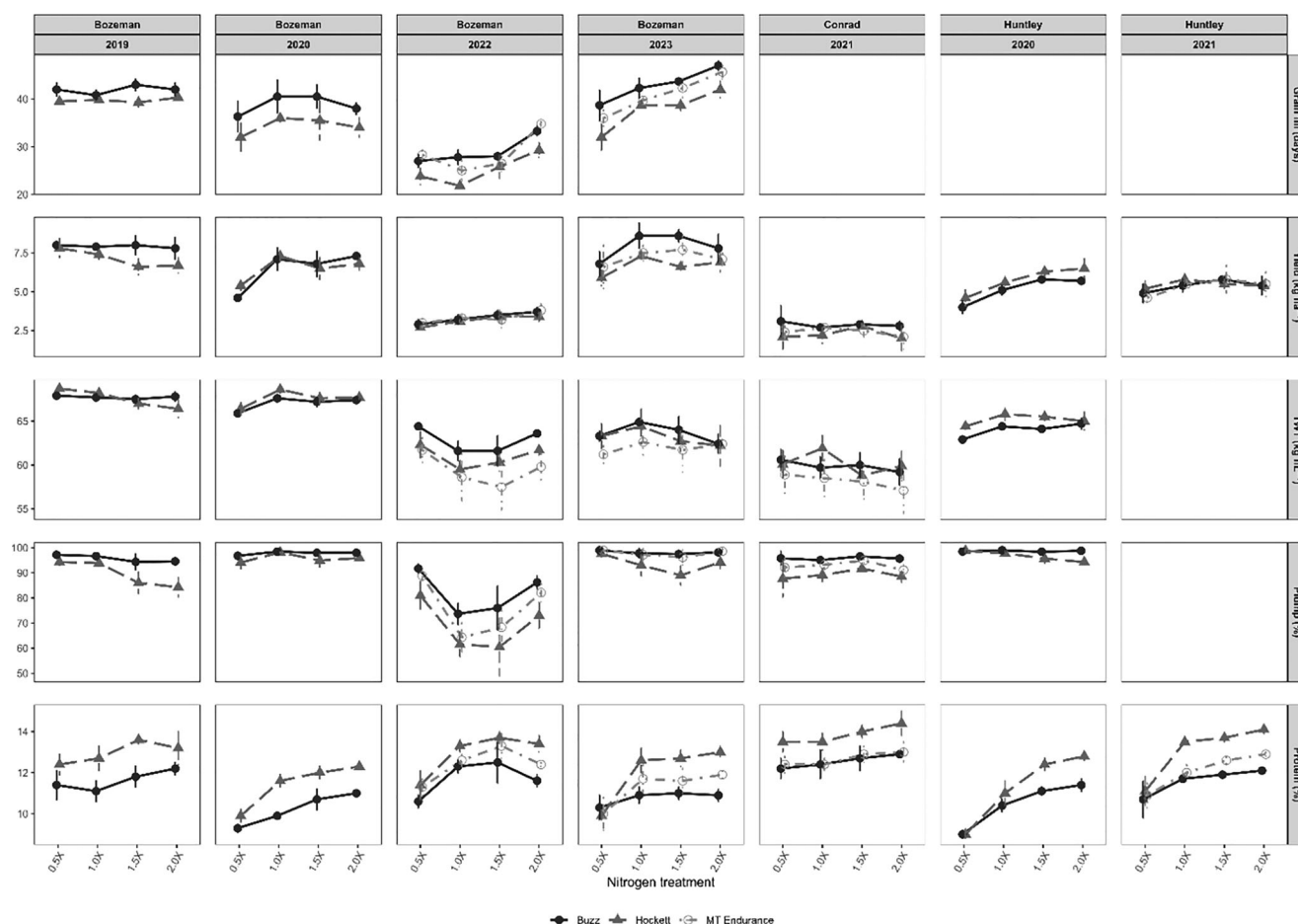


FIGURE 2 Nitrogen rate responses of agronomic traits in barley cultivars across location-years. Traits include grain fill duration, grain yield, test weight (TWT), plump kernels, and grain protein. Points represent treatment means and error bars indicate ± 1 standard deviation.

3.7 | Environmental variation led to excess 1.0x N and revealed contrasting cultivar stability

Environmental variability across years and locations complicated estimation of yield potential and increased the likelihood that the 1.0x N treatment exceeded crop demand. Among the seven field trials, four location-years were identified in which environmental conditions reduced realized yield potential, resulting in excess available N at the 1.0x rate (Table 1) and leading to increased grain protein concentration and reduced kernel plumpness.

In Bozeman 2022, reduced precipitation during June and July combined with elevated temperatures in July coincided with increased grain protein concentration and reduced kernel plumpness (Figure 4). Under these conditions, Hockett exceeded the AMBA protein threshold ($>13\%$) and fell below the plump kernel specification ($<90\%$), disqualifying it from both all-malt and adjunct malt use (Figure 4). Buzz and MT Endurance maintained acceptable grain protein concentrations but did not meet the plump kernel threshold except at the lowest N treatment (Figure 4).

In Conrad 2021, below-average precipitation and elevated temperatures during June and July similarly resulted in excess available N relative to realized yield potential (Table 1). Under these conditions, Hockett exceeded the protein threshold and failed to meet plump kernel standards, whereas Buzz and MT Endurance met AMBA specifications for both traits (Figure 4).

At Huntley, apparent overfertilization occurred in both 2020 and 2021 despite irrigation; however, only the 2021 season experienced elevated maximum temperatures in June and July (Table 2). In 2020, all cultivars met AMBA standards for grain protein and plump kernels (Figure 4). In contrast, in 2021, Hockett exceeded the protein threshold, whereas Buzz and MT Endurance remained within acceptable limits (Figure 4). Data on plump kernels were not available for this year.

In the remaining Bozeman location-years (2019, 2020, and 2023), where the 1.0x N rate aligned more closely with realized yield potential, all cultivars met AMBA specifications for both grain protein and kernel plumpness (Table S7).

Across environments where the 1.0x N rate exceeded crop demand, Hockett more frequently exceeded the protein

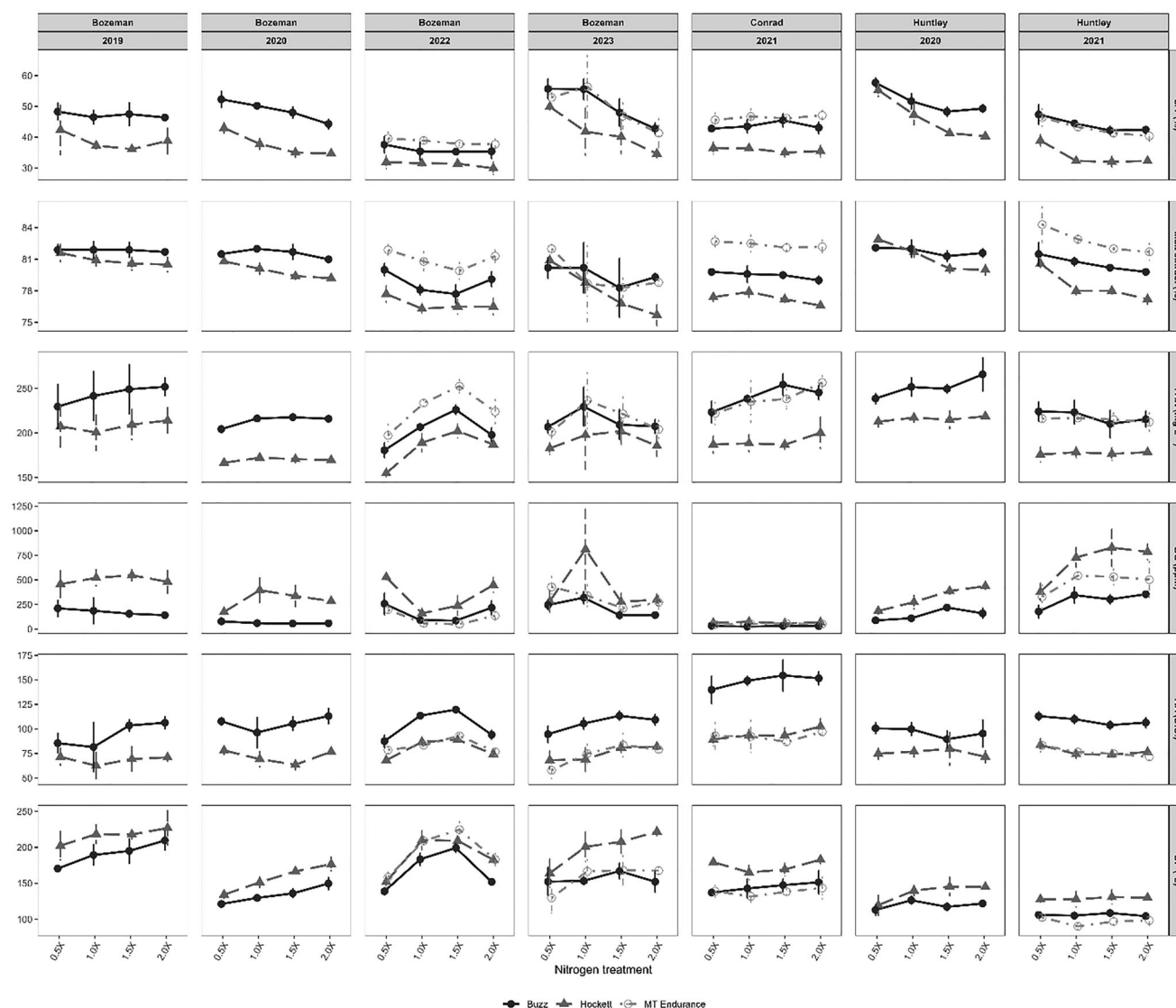


FIGURE 3 Nitrogen rate responses of malt quality traits in barley cultivars across location-years. Traits include soluble-to-total protein ratio (S/T), malt extract, free amino nitrogen (FAN), beta-glucan (BG), alpha-amylase (AA), and diastatic power (DP). Points represent treatment means and error bars indicate ± 1 standard deviation.

threshold and exhibited larger reductions in plumpness relative to Buzz and MT Endurance, indicating lower malt quality stability when available N exceeded realized yield potential.

4 | DISCUSSION

4.1 | Genetic differences in grain fill duration and their association with grain quality traits

Differences in grain quality observed among cultivars were consistent with allelic variation at the three stay-green QTLs linked to grain fill duration and grain composition (Table S1). KASP genotyping confirmed that Buzz carries stay-green alleles at *QGFhd-2H* and *QGFmt-6H*, MT Endurance carries

alleles at *QGFmt-6H* and *QGFhd-7H*, and Hockett carries the stay-green allele only at *QGFhd-7H* (Table S1). These loci collocate with genomic regions previously associated with grain protein concentration, kernel plumpness, and developmental timing, suggesting a shared physiological mechanism influencing both assimilate duration and partitioning between starch and protein (Williams et al., 2024).

The *QGFhd-2H* stay-green allele in Buzz is associated with earlier heading and extended grain fill duration and has been linked to variation in protein concentration and plumpness (Alqudah et al., 2014; Dang et al., 2022; Pauli et al., 2015; Williams et al., 2024). The *QGFmt-6H* allele, present in both Buzz and MT Endurance, was associated with later maturity, extended grain fill duration, improved plumpness, and reduced grain protein concentration (Williams et al., 2024).

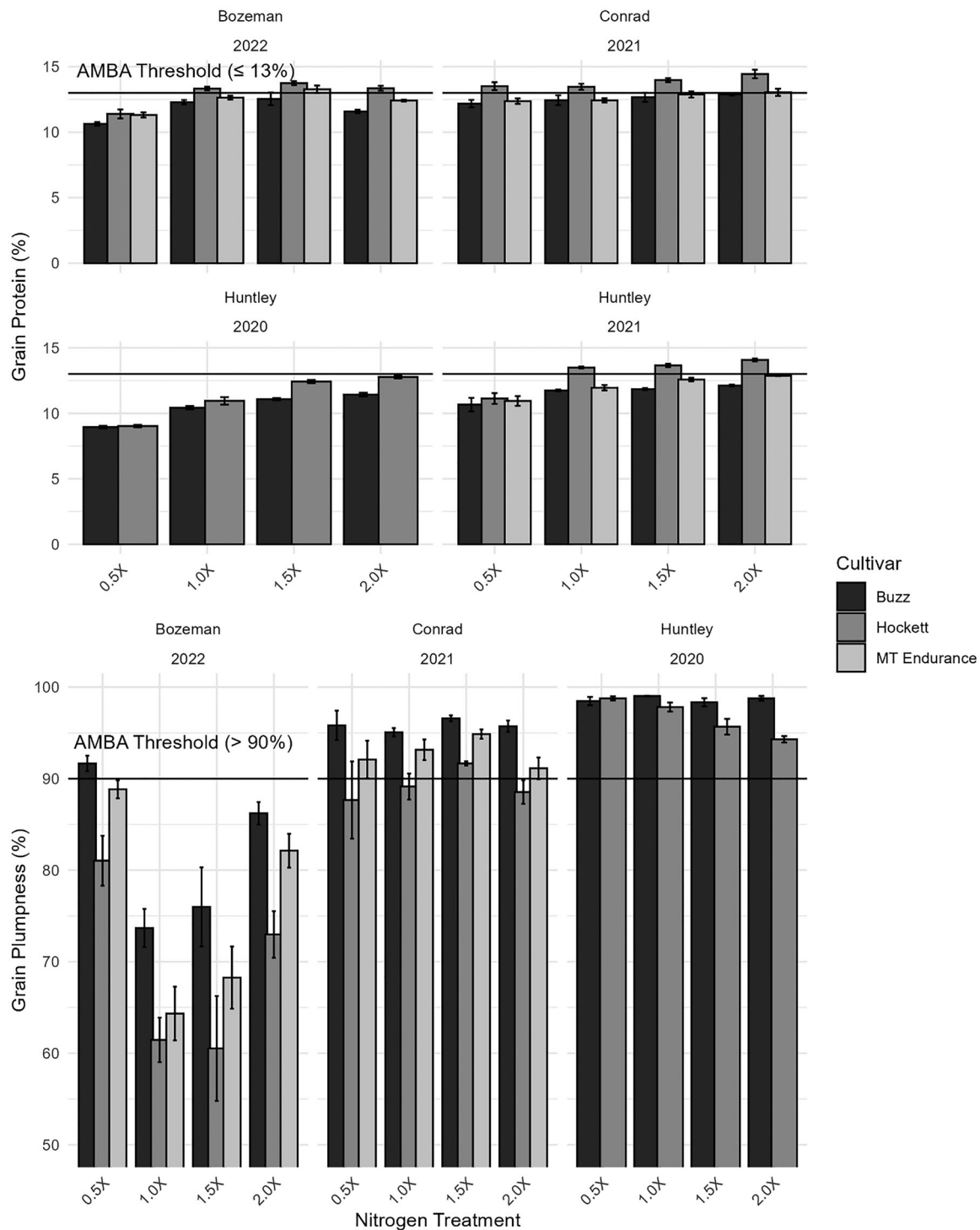


FIGURE 4 Mean grain protein concentration and kernel plumpness for each cultivar $\times$ nitrogen treatment combination in location-years where the 1.0x nitrogen treatment exceeded realized yield potential. Error bars indicate ± 1 SE. Horizontal reference lines indicate American Malting Barley Association (AMBA) malt quality thresholds of $\leq 13\%$ grain protein and $> 90\%$ plump kernels retained on a 6/64-in. sieve (AMBA, 2025).

This region contains the *HvNAM1* NAC transcription factor gene, previously linked to delayed senescence, reduced protein concentration, and increased kernel weight (Alptekin et al., 2021; Distelfeld et al., 2008; See et al., 2002). The *QGfhd-7H* allele, shared by MT Endurance and Hockett, has been associated with earlier heading and an extension of grain

fill duration (Alqudah et al., 2014; Dang et al., 2022; Williams et al., 2024).

Phenotypic differences among cultivars reflected the number and combination of stay-green alleles. Across environments, Buzz and MT Endurance exhibited longer grain fill duration, lower grain protein, and greater kernel plumpness

relative to Hockett (Table S7), consistent with the expected effects of prolonged photosynthetic activity during grain development. Genome-wide SNP genotyping showed that Buzz and Hockett differed at 13% of the markers and MT Endurance and Hockett differed at 21%, indicating that phenotypic differences reflect both stay-green QTL effects and additional genetic variation.

Extended grain fill duration is expected to promote starch accumulation relative to nitrogen remobilization, improving the balance between carbohydrate deposition and protein concentration. Previous research has demonstrated that post-anthesis assimilates primarily contribute to grain size, while N remobilized from vegetative tissues largely determines grain protein concentration (Barmeier et al., 2021). In this context, the combination of *QGFhd-2H* and *QGFmt-6H* stay-green alleles in Buzz may enhance assimilate duration during grain development, supporting increased kernel plumpness while moderating protein concentration across fertility levels and environments.

MT Endurance carries the *QGFmt-6H* and *QGFhd-7H* stay-green alleles and exhibited an intermediate phenotype relative to Buzz and Hockett, including moderately extended grain fill duration, reduced protein concentration relative to Hockett, and improved plumpness (Table S7). The *QGFhd-7H* locus has been associated with earlier heading and moderate extension of grain fill duration (Williams et al., 2024), and both MT Endurance and Hockett carry this allele. However, MT Endurance consistently headed earlier than Hockett across Bozeman location-years (Table S7), indicating that the presence of the stay-green allele at *QGFhd-7H* alone does not fully explain differences in developmental timing. These patterns suggest that additional loci influence heading date in MT Endurance, either independently or through interactions with *QGFhd-7H*, highlighting that additional genetic factors likely contribute to agronomic and malt quality variation.

Differences among cultivars were also observed for malt extract, where MT Endurance consistently exhibited the highest malt extract, followed by Buzz, while Hockett typically had the lowest extract values (Figure 3; Table S9). Across location-years, protein content was negatively correlated with malt extract (Table S5), consistent with previous reports (Cai et al., 2013; Edney et al., 2012; Halstead et al., 2022). The reduced protein concentration associated with the *QGFmt-6H* stay-green allele may partly contribute to increased malt extract potential. However, MT Endurance exhibited greater malt extract than Buzz in several environments despite similar or slightly higher protein concentrations (Tables S8 and S9), suggesting that loci beyond the evaluated stay-green QTLs may influence malt extract potential.

Collectively, these findings support the value of stay-green-associated alleles for improving grain quality under variable environments and fertility conditions through extended grain

fill duration. Further work using near-isogenic lines or expanded genomic analyses may help clarify the individual and combined effects of stay-green alleles on grain development and malting performance.

4.2 | Prolonged grain fill supports grain quality consistency under increased fertility in variable environments

Barley growers in major production regions typically apply N at recommended rates but face uncertainty due to environmental variability. When precipitation is below the long-term average or temperatures are elevated during grain fill, realized yield potential may be reduced relative to preseason expectations, increasing the likelihood that applied N exceeds crop demand. Under these conditions, excessive N frequently results in elevated grain protein concentration and reduced kernel plumpness, increasing the risk that grain fails to meet malting specifications (Jackson, 2000; McKenzie et al., 2005; O'Donovan et al., 2011). In the present study, grain protein concentration was strongly influenced by genotype, environment, and N management, consistent with previous findings (Edney et al., 2012; Halstead et al., 2022; O'Donovan et al., 2011). Across environments and N treatments, protein concentration was negatively associated with key quality traits, including plumpness, TWT, and malt extract, reinforcing grain protein as a major driver of malt quality variation (Cai et al., 2013; Edney et al., 2012; Halstead et al., 2022; O'Donovan et al., 2011).

Extended grain fill may help maintain kernel development under conditions where N availability exceeds realized yield potential. Across Bozeman location-years, grain fill duration showed positive associations with plumpness in several environments, although the strength of this relationship varied among years (Table S5). Kernel plumpness was consistently negatively associated with grain protein concentration across multiple environments (Table S5), supporting its role as a key trait linking N-driven protein increases to malting quality outcomes. Together, these relationships suggest that prolonged grain fill can help sustain starch deposition and kernel development under variable fertility conditions.

Across the seven field trials, four location-years were identified in which the 1.0x N treatment resulted in excess available N relative to realized yield potential (Table 1). The protein-plumpness trade-off was most evident in environments where N inputs exceeded crop demand and coincided with late season stress conditions. For example, both Huntley 2020 and 2021 received N inputs exceeding realized yield potential, but only 2021 experienced mean maximum temperatures in June and July above the long-term average (Table 2). Under these conditions, Hockett exceeded the 13% protein threshold at N rates above 0.5X (Figure 4)

and exhibited reduced malt extract, lower S/T, and elevated BG concentrations (Table S9), responses commonly associated with excessive N availability during grain development (Mahalingam & Bregitzer, 2018; Mangelsen et al., 2011; Wallwork et al., 1998). In contrast, Buzz and MT Endurance maintained protein concentrations below 13% across most environments and N treatments, indicating reduced sensitivity to increases in available N. Although plumpness occasionally fell below the AMBA threshold, both cultivars consistently maintained larger kernel size than Hockett under elevated N.

These results support the role of extended grain fill duration in moderating the negative effect of excess N on malt quality traits. Prolonged photosynthetic activity during grain fill may allow greater carbohydrate deposition relative to nitrogen accumulation, improving the balance between starch and protein under variable environmental conditions. Even in years when environmental variability reduced realized yield potential and increased the likelihood of excess N, cultivars with extended grain fill more frequently maintained acceptable protein levels and kernel plumpness. Together, these findings indicate that stay-green-associated traits may improve stability of malting quality across environments where mismatches between preseason fertility decisions and in-season growing conditions occur.

4.3 | Trade-offs and limitations of stay-green cultivars

Buzz and MT Endurance consistently exhibit favorable malt quality traits, including lower protein concentration, higher kernel plumpness (Table S7), increased malt extract, and reduced BG content (Table S9). However, these advantages were accompanied by differences in enzymatic profiles relative to Hockett. Across location-years and N rates, Buzz and MT Endurance tended to exhibit elevated AA activity and higher S/T, alongside reduced DP (Table S9), indicating potential shifts in malt modification characteristics associated with extended grain fill phenotypes.

During malting, hydrolytic enzymes are activated to degrade starch, proteins, and cell walls components, and the degree of malt modification reflects the extent of this biochemical conversion (Bamforth, 2009; Cu et al., 2016). Elevated AA and S/T in Buzz and MT Endurance suggest more extensive modification under standard malting conditions, which may influence brewhouse performance depending on process requirements. Since genotype $\times$ modification relationships can influence malt performance traits (Herb et al., 2017), maltster may need to adjust steeping or germination conditions to optimize enzyme balance and modification levels for cultivars expressing extended grain fill phenotypes.

Grain intended for adjunct brewing typically requires high malt extract and sufficient enzymatic capacity to convert starch from both malted and unmalted grain sources (Eagles et al., 1995). In the present study, Buzz and MT Endurance combined favorable protein and extract characteristics but exhibited lower DP relative to Hockett (Tables S8 and S9). Although elevated AA activity may enhance starch hydrolysis, reduced DP may limit total enzymatic capacity for starch conversion, potentially influencing wort fermentability (Cu et al., 2016; Evans et al., 2005). These differences may be most relevant in adjunct brewing systems, where efficient conversion of non-malt starch relies on adequate enzymatic strength.

Extended grain fill and delayed maturity may also introduce agronomic considerations. Prolonged phenology increases the duration that grain remains exposed in the field prior to harvest, potentially increasing vulnerability to late-season precipitation events that promote preharvest sprouting (Rooney et al., 2023). Extended phenology may also increase exposure to late-season weather events such as hail or wind damage, as well as lodging risk when harvest is delayed (Gilbertson & Hockett, 1979; Jedel & Helm, 1991). However, under dryland conditions in this study, differences in maturity date between Buzz and MT Endurance relative to Hockett were modest, typically only a few days (Table S7), indicating limited increases in late-season exposure risk. The modest increase in late-season exposure risk must therefore be weighed against the benefits of improved tolerance to heat and water limitation during grain fill, as cultivars with extended grain fill maintained favorable agronomic and malt quality performance across environments in this study.

From a breeding and production perspective, these results highlight the importance of balancing agronomic adaptation with end use processing requirements. Extended grain fill cultivars improved stability of protein concentration and kernel plumpness across variable fertility and environments but also exhibited differences in enzyme balance that may influence malting performance. Buzz and MT Endurance showed evidence of greater modification than Hockett when processed under identical conditions, as indicated by higher S/T and FAN, and lower BG (Table S9). Consequently, cultivar adoption may benefit from coordinated evaluation among breeders, producers, maltsters, and brewers to ensure optimal performance across the grain supply chain.

5 | CONCLUSION

Results from this multiyear, multilocation study demonstrate that stay-green-associated variation in grain fill duration can improve key malting quality traits across environments with variable climate and N availability. Extended grain fill dura-

tion was consistently associated with a more favorable balance between starch accumulation and protein concentration, supporting kernel plumpness and reducing the risk of excessive grain protein when N inputs exceeded realized yield potential. Grain protein and kernel plumpness are primary determinants of whether producers meet malting specifications and receive price premiums; therefore, incorporation of stay-green-associated alleles into elite malting germplasm may improve reliability of malt-quality attainment in semiarid production systems characterized by high seasonal variability.

These results extend prior work by demonstrating that stay-green-related grain fill dynamics can help stabilize malting quality not only under environmental limitations, but also under variable N management where preseason decisions do not always align with in-season yield potential. In environments where yield potential is reduced by precipitation or temperature variability, extended grain fill may help maintain kernel development and reduce the likelihood that excess available N results in grain failing to meet malting quality specifications.

Several limitations should be considered when interpreting these findings. The QTLs evaluated represent relatively broad genomic regions, and observed relationships should not be interpreted as fully causative. Although responses varied among environments, extended grain fill was consistently associated with improved attainment of key malting quality traits under conditions where N availability exceeded crop demand. Future work evaluating individual and stacked stay-green QTLs in defined genetic backgrounds, together with broader validation across production environments, will strengthen inference regarding allele-specific effects and support the development of cultivars with improved malt quality stability under variable climatic and fertility conditions.

AUTHOR CONTRIBUTIONS

Trevor A. Palone: Data curation; formal analysis; visualization; writing—original draft. **Clint Beiermann:** Investigation; resources. **Patrick M. Carr:** Conceptualization; investigation; methodology; resources; writing—review and editing. **Joseph Jensen:** Investigation; writing—review and editing. **Greg Lutgen:** Investigation; resources. **Kent McVay:** Conceptualization; investigation; methodology. **Qasim A. Khan:** Investigation; resources. **Teigen Nelson:** Investigation. **Hannah Uhlmann:** Data curation; investigation; resources. **Jessica L. Williams:** Investigation; writing—review and editing. **Jamie D. Sherman:** Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon request.

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REFERENCES

- Alptekin, B., Mangel, D., Pauli, D., Blake, T., Lachowicz, J., Hoogland, T., Fischer, A., & Sherman, J. (2021). Combined effects of a glycine-rich RNA-binding protein and a NAC transcription factor extend grain fill duration and improve malt barley agronomic performance. *Theoretical and Applied Genetics*, 134(1), 351–366. <https://doi.org/10.1007/s00122-020-03701-1>
- Alqudah, A. M., Sharma, R., Pasam, R. K., Graner, A., Kilian, B., & Schnurbusch, T. (2014). Genetic dissection of photoperiod response based on GWAS of pre-anthesis phase duration in spring barley. *PLoS ONE*, 9(11), e113120. <https://doi.org/10.1371/journal.pone.0113120>
- AMBA. (2025). *Guidelines for malting barley breeders*. <https://ambainc.org/news-details.php?id=63d2780a0c948>
- Bamforth, C. W. (2009). Current perspectives on the role of enzymes in brewing. *Journal of Cereal Science*, 50(3), 353–357. <https://doi.org/10.1016/j.jcs.2009.03.001>
- Barmeier, G., Hu, Y., & Schmidhalter, U. (2021). Partitioning and translocation of dry matter and nitrogen during grain filling in spring barley varieties and their roles in determining malting quality. *Frontiers in Plant Science*, 12, 722871. <https://doi.org/10.3389/fpls.2021.722871>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1). <https://doi.org/10.18637/jss.v067.i01>
- Bayer, M. M., Rapazote-Flores, P., Ganai, M., Hedley, P. E., Macaulay, M., Plieske, J., Ramsay, L., Russell, J., Shaw, P. D., Thomas, W., & Waugh, R. (2017). Development and evaluation of a barley 50k iSe-

- lect SNP array. *Frontiers in Plant Science*, 8, 1792. <https://doi.org/10.3389/fpls.2017.01792>
- Bohačenko, I., Psota, V., Hartmann, J., & Musilová, M. (2021). Combined effect of high temperature and drought on yield and malting quality of barley. *Czech Journal of Food Sciences*, 39(1), 17–22. <https://doi.org/10.17221/146/2019-cjfs>
- Cai, S., Yu, G., Chen, X., Huang, Y., Jiang, X., Zhang, G., & Jin, X. (2013). Grain protein content variation and its association analysis in barley. *BMC Plant Biology*, 13(1), 35. <https://doi.org/10.1186/1471-2229-13-35>
- Comadran, J., Kilian, B., Russell, J., Ramsay, L., Stein, N., Ganai, M., Shaw, P., Bayer, M., Thomas, W., Marshall, D., Hedley, P., Tondelli, A., Pecchioni, N., Francia, E., Korzun, V., Walther, A., & Waugh, R. (2012). Natural variation in a homolog of *Antirrhinum CENTRORADIALIS* contributed to spring growth habit and environmental adaptation in cultivated barley. *Nature Genetics*, 44(12), 1388–1392. <https://doi.org/10.1038/ng.2447>
- Cu, S. T., March, T. J., Stewart, S., Degner, S., Coventry, S., Box, A., Stewart, D., Skadhauge, B., Burton, R. A., Fincher, G. B., & Eglinton, J. (2016). Genetic analysis of grain and malt quality in an elite barley population. *Molecular Breeding*, 36(9), 129. <https://doi.org/10.1007/s11032-016-0554-z>
- Dang, V. H., Hill, C. B., Zhang, X.-Q., Angessa, T. T., Mcfawn, L.-A., & Li, C. (2022). Multi-locus genome-wide association studies reveal novel alleles for flowering time under vernalisation and extended photoperiod in a barley MAGIC population. *Theoretical and Applied Genetics*, 135(9), 3087–3102. <https://doi.org/10.1007/s00122-022-04169-x>
- Distelfeld, A., Korol, A., Dubcovsky, J., Uauy, C., Blake, T., & Fahima, T. (2008). Colinearity between the barley grain protein content (GPC) QTL on chromosome arm 6HS and the wheat Gpc-B1 region. *Molecular Breeding*, 22(1), 25–38. <https://doi.org/10.1007/s11032-007-9153-3>
- Eagles, H., Bedgood, A., Panozzo, J., & Martin, P. (1995). Cultivar and environmental effects on malting quality in barley. *Australian Journal of Agricultural Research*, 46(5), 831–844. <https://doi.org/10.1071/ar9950831>
- Edney, M. J., O'Donovan, J. T., Turkington, T. K., Clayton, G. W., McKenzie, R., Juskiw, P., Lafond, G. P., Brandt, S., Grant, C. A., Harker, K. N., Johnson, E., & May, W. (2012). Effects of seeding rate, nitrogen rate and cultivar on barley malt quality. *Journal of the Science of Food and Agriculture*, 92(13), 2672–2678. <https://doi.org/10.1002/jsfa.5687>
- Evans, D. E., Collins, H., Eglinton, J., & Wilhelmson, A. (2005). Assessing the impact of the level of diastatic power enzymes and their thermostability on the hydrolysis of starch during wort production to predict malt fermentability. *Journal of the American Society of Brewing Chemists*, 63(4), 185–198. <https://doi.org/10.1094/asbcj-63-0185>
- Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). Sage. <https://www.john-fox.ca/Companion/>
- Gilbertson, K., & Hockett, E. (1979). The effect of hail damage on barley. *Canadian Journal of Plant Science*, 59(4), 1147–1152. <https://doi.org/10.4141/cjps79-177>
- Gous, P. W., Hasjim, J., Franckowiak, J., Fox, G. P., & Gilbert, R. G. (2013). Barley genotype expressing “stay-green”-like characteristics maintains starch quality of the grain during water stress condition. *Journal of Cereal Science*, 58(3), 414–419. <https://doi.org/10.1016/j.jcs.2013.08.002>
- Gous, P. W., Hickey, L., Christopher, J. T., Franckowiak, J., & Fox, G. P. (2015). Discovery of QTL for stay-green and heat-stress in barley (*Hordeum vulgare*) grown under simulated abiotic stress conditions. *Euphytica*, 207(2), 305–317. <https://doi.org/10.1007/s10681-015-1542-9>
- Halstead, M., Morrissy, C., Fisk, S., Fox, G., Hayes, P., & Carrijo, D. (2022). Barley grain protein is influenced by genotype, environment, and nitrogen management and is the major driver of malting quality. *Crop Science*, 63(1), 115–127. <https://doi.org/10.1002/csc2.20842>
- Harrell, F. E., Jr. (2020). Hmisc: Harrell Miscellaneous (R package version 5.2-5). <https://CRAN.R-project.org/package=Hmisc>
- Herb, D., Filichkin, T., Fisk, S., Helgersson, L., Hayes, P., Benson, A., Vega, V., Carey, D., Thiel, R., Cistue, L., Jennings, R., Monsour, R., Tynan, S., Vinkemeier, K., Li, Y., Nguyen, A., Onio, A., Meints, B., Moscou, M., ... Thomas, W. (2017). Malt modification and its effects on the contributions of barley genotype to beer flavor. *Journal of the American Society of Brewing Chemists*, 75(4), 354–362. <https://doi.org/10.1094/asbcj-2017-4976-01>
- Jackson, G. (2000). *Nitrogen fertilization of dryland malt barley for yield and quality*. Montana State University. <https://landresources.montana.edu/fertilizerfacts/documents/FF24NDrylandMaltBarley.pdf>
- Jedel, P. E., & Helm, J. H. (1991). Lodging effects on a semidwarf and two standard barley cultivars. *Agronomy Journal*, 83(1), 158–161. <https://doi.org/10.2134/agronj1991.00021962008300010036x>
- Kamal, N. M., Gorafi, Y. S. A., Abdelrahman, M., Abdellatif, E., & Tsujimoto, H. (2019). Stay-green trait: A prospective approach for yield potential, and drought and heat stress adaptation in globally important cereals. *International Journal of Molecular Sciences*, 20(23), 5837. <https://doi.org/10.3390/ijms20235837>
- Knapp, C. N., Kluck, D. R., Guntenspergen, G., Ahlering, M. A., Aimone, N. M., Bamzai-Dodson, A., Basche, A., Byron, R. G., Conroy-Ben, O., Haggerty, M. N., Haigh, T. R., Johnson, C., Boustead, B. M., Mueller, N. D., Ott, J. P., Paige, G. B., Ryberg, K. R., Schuurman, G. W., & Tangen, S. G. (2023). Northern Great Plains. In A. R. Crimmins, C. W. Avery, D. R. Easterling, K. E. Kunkel, B. C. Stewart, & T. K. Maycock (Eds.), *Fifth National Climate Assessment* (pp. 25–1–25–41). U.S. Global Change Research Program. https://www.fs.usda.gov/rm/pubs_journals/2024/rmrs_2024_kluck_d001.pdf
- Lenth, R., & Piskowski, J. (2023). Emmeans: Estimated Marginal Means, aka Least-Squares Means (R package version 2.0.3). <https://rvinth.github.io/emmeans/>
- Mahalingam, R., & Bregitzer, P. (2018). Impact on physiology and malting quality of barley exposed to heat, drought and their combination during different growth stages under controlled environment. *Physiologia Plantarum*, 165(2), 277–289. <https://doi.org/10.1111/ppl.12841>
- Mangelsen, E., Kilian, J., Harter, K., Jansson, C., Wanke, D., & Sundberg, E. (2011). Transcriptome analysis of high-temperature stress in developing barley caryopses: Early stress responses and effects on storage compound biosynthesis. *Molecular Plant*, 4(1), 97–115. <https://doi.org/10.1093/mp/ssq058>
- McKenzie, R. H., Middleton, A. B., & Bremer, E. (2005). Fertilization, seeding date, and seeding rate for malting barley yield and quality in southern Alberta. *Canadian Journal of Plant Science*, 85(3), 603–614. <https://doi.org/10.4141/p04-152>

- McVay, K., Burrows, M., Jones, C., Wanner, K., & Menalled, F. (2017). *Montana barley production guide* (EB0186). Montana State University Extension.
- NOAA NCEI. (2021). *U.S. climate normals*. <https://www.ncei.noaa.gov/products/land-based-station/us-climate-normals/>
- O'Donovan, J. T., Turkington, T. K., Edney, M. J., Clayton, G. W., McKenzie, R. H., Juskiw, P. E., Lafond, G. P., Grant, C. A., Brandt, S., Harker, K. N., Johnson, E. N., & May, W. E. (2011). Seeding rate, nitrogen rate, and cultivar effects on malting barley production. *Agronomy Journal*, 103(3), 709–716. <https://doi.org/10.2134/agronj2010.0490>
- Pauli, D., Brown-Guedira, G., & Blake, T. K. (2015). Identification of malting quality QTLs in advanced generation breeding germplasm. *Journal of the American Society of Brewing Chemists*, 73(1), 29–40. <https://doi.org/10.1094/asbcj-2015-0129-01>
- R Core Team. (2024). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.r-project.org/>
- Rooney, T. E., Sweeney, D. W., Kunze, K. H., Sorrells, M. E., & Walling, J. G. (2023). Malting quality and preharvest sprouting traits are genetically correlated in spring malting barley. *Theoretical and Applied Genetics*, 136(3), 59. <https://doi.org/10.1007/s00122-023-04257-6>
- See, D., Kanazin, V., Kephart, K., & Blake, T. (2002). Mapping genes controlling variation in barley grain protein concentration. *Crop Science*, 42(3), 680–685. <https://doi.org/10.2135/cropsci2002.6800>
- Thomas, H., & Ougham, H. (2014). The stay-green trait. *Journal of Experimental Botany*, 65(14), 3889–3900. <https://doi.org/10.1093/jxb/eru037>
- Turner, H. M., Elmore, L., Walling, J., Lachowicz, J., Mangel, D., Fischer, A., & Sherman, J. (2019). Effect of steeping regime on barley malt quality and its impacts on breeding program selection. *Journal of the American Society of Brewing Chemists*, 77(4), 267–281. <https://doi.org/10.1080/03610470.2019.1629794>
- USDA NASS. (2025a). *Quick stats*. <https://quickstats.nass.usda.gov/results/A08C0C14-0A6F-38BE-989E-E4154C2A3B47>
- USDA NASS. (2025b). *Quick stats*. <https://quickstats.nass.usda.gov/results/01447186-1B10-3BB3-B6A5-59417BC6D7D5>
- Wallwork, M. A. B., Logue, S. J., MacLeod, L. C., & Jenner, C. F. (1998). Effect of high temperature during grain filling on starch synthesis in the developing barley grain. *Functional Plant Biology*, 25(2), 173–181. <https://doi.org/10.1071/pp97084>
- Whitlock, C., Cross, W., Maxwell, B., Silverman, N., & Wade, A. A. (2017). *2017 Montana climate assessment*. Montana Institute on Ecosystems. <https://doi.org/10.15788/m2ww8w>
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. <https://ggplot2.tidyverse.org>
- Williams, J. L., Lamb, P. F., Lutgen, G., Lachowicz, J., Cook, J. P., Jensen, J., Bourgault, M., & Sherman, J. D. (2024). QTL mapping reveals malt barley quality improvement in two dryland environments associated with extended grain fill and seminal root traits. *Crop Science*, 64(4), 2126–2142. <https://doi.org/10.1002/csc2.21253>
- Williams, J. L., Sherman, J. D., Lamb, P., Cook, J., Lachowicz, J. A., & Bourgault, M. (2022). Relationships between roots, the stay-green phenotype, and agronomic performance in barley and wheat grown in semi-arid conditions. *The Plant Phenome Journal*, 5(1), e220050. <https://doi.org/10.1002/ppj2.20050>
- Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed Research*, 14(6), 415–421. <https://doi.org/10.1111/j.1365-3180.1974.tb01084.x>

SUPPORTING INFORMATION

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

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