Effects of Genotype and Environment on Productivity and Quality in Californian Malting Barley

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Core Ideas

1. The Location x Year interaction explained the majority of the variance for GPC and yield.
2. Added nitrogen fertilizer levels at tillering accounted for 5% of the variance in Grain Protein Content.
3. Y, Genotype and L x Y explained the largest variance in onset, peak and offset GT, respectively.
4. The 2020-21 samples formed partially distinct clusters, segregated by high thins and onset GT.
5. Plumper grains had a lower onset GT but a larger difference between peak and offset GT.

ASBC, American Society of Brewing Chemists; GT, Gelatinization temperature; G, Genotype; GPC, Grain Protein Content; LME, Linear Mixed Effects; L, Location; N, Nitrogen; UC, University of California; Y, Year

ABSTRACT

Malting barley productivity and grain quality are of critical importance to the malting and brewing industry. In this study, we analyzed two experiments: a multi-environment variety trial and a nitrogen management trial. In the first experiment, we analyzed 12 malting barley genotypes across eight locations in California and three years (2017-18, 2018-19 and 2020-21). The effects of genotype (G), location (L), year (Y) and their interactions were assessed on grain yield (kg ha^{-1}), grain protein content (GPC; %), individual-grain weight, grain size (plump and thin; %), onset gelatinization temperature (GT), peak GT, offset GT, difference between onset

and peak GT and difference between peak and offset GT. L, Y and their interaction explained the largest variance for all traits except peak GT and difference between onset and peak GT, for which G explained the largest variance. The 2020-21 samples formed partially distinct clusters in principal component analysis, mainly discriminated by high percentage of thin grains and high onset GT. In the second experiment, we analyzed a dataset with two genotypes across three locations (with varying nitrogen fertilizer levels) from the 2016-17 season to assess the effect of added nitrogen on the same traits. Added nitrogen at tillering explained 18% of variance in the difference between onset and peak GT, and 5% of the variance in GPC, but was minimal for all other traits, with the largest variance explained by location and genotype. These findings illustrate the key roles of G, L and Y in determining malting barley productivity and quality.

INTRODUCTION

Grain of malting barley is typically composed of 50 to 68% starch (Newman and Newman, 1992; You and Izydorczyk, 2007; Patindol et al., 2012), of which 70 to 80% is typically amylopectin and the remaining 20 to 30% is amylose (Greenwood and Thomson, 1959; Vasanthan and Bhatty, 1996; Izydorczyk et al., 2001; Källman et al., 2015). Amylose consists of linear chains of glucose units that are linked by α (1-4) glycosidic bonds. Amylopectin is a branched polymer composed of glucose units linked by both α (1-4) and α (1-6) glycosidic bonds.

Barley starch composition plays an important role during the mashing stage of the brewing process (Briggs, 1998). Mashing is carried out as part of the brewing process to extract sugars from the grain into the wort (i.e., infusion of malt), which is later fermented by yeasts to produce beer. During mashing, starch is hydrolyzed to fermentable sugars such as maltose and maltotriose. However, for enzymes to efficiently hydrolyze starch, starch must be gelatinized.

There is a range in which the starch granules gelatinize indicated by an onset, peak and offset gelatinization temperature (GT). In high-quality barley, the start of solubilization would be as low as 56°C (onset) and end around 65°C (offset). However, in barley that has been stressed due to high temperature or drought during the grain-fill period, the temperature range could be higher (Myllärinen et al., 1998; Gous et al., 2015). Hydrolysis is characterized by swelling of the starch granules within the endosperm and further gelatinization which has been shown to typically occur between 60°C and 65°C in malting barley. This GT range does not change substantially between raw and malted barley, but there is a slight increase due to the malting process (Langenaeken et al., 2019). Two families of enzymes, namely α and β-amylases, also play an important role in catalyzing this starch gelatinization. However, it has been shown that if the starch GT exceeds 65°C, β-amylase is rapidly inactivated, which has been found to reduce brewing efficiency (Evans et al., 2003). Hence, starch GT can serve as an indicator of malting barley quality and brewing performance.

Starch GT range in malting barley has been shown to be affected by several factors such as starch granule size (Karlsson et al., 1983), starch granule packing (Fox et al., 2007), amylose to amylopectin ratios, total amylose content (Fredriksson et al., 1998; Källman et al., 2015), protein content as a percentage of grain weight (Wenwen et al., 2019) and grain weight (Kandic et al., 2019). Protein levels could influence GT due to starch-protein interactions in the endosperm matrix, which inhibit the swelling of starch granules during mashing (Wenwen et al., 2019). Finally, a positive correlation between grain weight and starch GT has also been reported (Kandic et al., 2019).

The barley belt is a term used to describe the primary barley production region in the US, spanning from Washington state in the west to North Dakota in the east (AMBA, 2022). In

California, malting barley growing regions are primarily located in the Sacramento and San Joaquin Valleys and south-Central Coast. In 2021, approximately 20% of malting barley produced in the state of California was grown in the Tulelake basin (Siskiyou County) as a rotation crop with potato, onion and alfalfa (Lindblad, 2022). In the Sacramento and San Joaquin Valleys and the Southern desert region, feed barley is grown predominantly as a rotation crop. These environments form part of the Central Valley and Imperial Valley of California with Mediterranean, semi-arid, and arid desert type climate profiles. Since malting barley is often grown as a rotation crop in a wide range of conditions (Kanter et al., 2021), it is critical to understand the impact of location on malting barley grain quality. Mediterranean climates are known for their temporal variability, driven by hot and dry summers and rainy winter spells with more frequent weather extremes (Nelsen and Lundy, 2020; Hochman et al., 2021). Despite these variable conditions, field crop acreage in California is projected to increase with increased advocacy of water-limited winter cropping systems in light of the recent Sustainable Groundwater Management Act (Peterson et al., 2022). However, the contributions of warming and increased incidents of drought to grain quality are still unknown.

The effects of genotype and environment (locations and/or years) have been previously studied for assessing malting barley quality in multiple barley production regions worldwide (Nielsen and Munck, 2003; Bantayehu, 2013; Przulj et al., 2014; Laidig et al., 2017) and in the US (Zhou et al., 2020; Choi et al., 2020). These studies have found the interaction between genotype and environment to play an important role in malting barley quality. However, the traits measured by these studies either were agronomic traits or were grain quality traits stipulated by the American Malting Barley Association. In order to understand the impact of G,

L and Y on downstream processing outcomes during brewing, it is important to consider more in-depth traits relating to starch gelatinization.

Studies have also found substantial variation in starch GT between multiple genotypes of malting barley (Gujral et al., 2013; Jaiswal et al., 2014; Pycia et al., 2015). Other studies have found that location (Fox et al., 2001), year (Przulj et al., 2014) and environmental factors such as drought stress (Gous et al., 2015) also affect starch GTs. However, these studies were not designed to include multiple locations, years and genotypes. In all cases, up to two out of the three were varied, while keeping the third variable constant. A multi-environment study has been conducted in California to assess yield performance in wheat (George and Lundy, 2019), but malting barley and quality traits have not received the same level of attention in this region to date. A major U.S genome-wide association study identified markers exhibiting significant associations with multiple malting barley quality traits; these instances of potential pleiotropy could make genomics-assisted selection for a full suite of quality traits challenging for breeders (Mohammadi et al., 2015).

This study aims to elucidate the complexity of maintaining malting barley productivity and quality in the context of inter-annual temperature and precipitation variability. In the two experiments analyzed herein, we assessed the following traits: grain yield (kg ha^{-1}), grain protein content (GPC; %), individual-grain weight, grain size (percentage of plump and thin grains), onset GT, peak GT, offset GT, difference between onset and peak GT, and difference between peak and offset GT. The first experiment examined whether genotype (G), location (L) and/or year (Y) play a more important role in affecting the aforementioned traits using samples from a multi-environment variety trial. Furthermore, correlations between these malting barley productivity and quality traits were also examined, with an aim to generate hypotheses at the

molecular/compositional level. The second experiment was focused on assessing the impact of added nitrogen (N) levels on the same traits. Variation in barley productivity and quality traits arising from G, L and/or Y combinations is leveraged herein to understand relationships among traits affecting end usability for maltsters and brewers.

MATERIALS AND METHODS

Barley

Multi-environment variety trial

The samples used were collected from variety trials conducted in 2017-2018, 2018-2019 and 2020-2021, by the University of California Small Grains Research team. These trials were planted in a randomized complete block design with four replications across all trial locations (UC-ANR, 2020; Nelsen and Lundy, 2020; Nelsen et al., 2021a). Grain from one out of the four replicates was used for further analysis. Twelve genotypes (9 varieties and 3 experimental lines) of two-row spring malting barley that were developed in the U.S. were included in this study, which were grown in eight field sites within California (Table S1; Figure S1). These plots were planted in the fall season (Table S1), in line with common agronomic practice in California (Jackson et al., 2006). These field trials were conducted in different areas of the state where malting barley is typically grown, with varying management practices based on the initial soil moisture and N levels at each location/year. Statewide yield statistics were obtained using the *tidyUSDA* package (Lindblad, 2022) in R 4.2.1 (R Core Team, 2020). The precipitation and temperature data were obtained from the California weather web-tool (Nelsen et al. 2021b).

N Fertilizer Management trial

The N fertilizer management trial was conducted in 2016-2017 with two genotypes across three locations. The samples were collected as previously described (Nelsen and Lundy, 2020). Briefly, sub-plots of each genotype-location combination were treated with varying N levels (0-150 kg ha^{-1}) applied at varying time periods (planting, tillering, or split application at planting and tillering) based on initial soil moisture and N levels.

For both trials, water levels were calculated as a sum of the irrigation and precipitation levels during the full growing season. The harvested grain was stored at room temperature for the first three to six months and then in temperature and humidity-controlled environments (< 10°C).

Barley flour

The raw barley samples were ground in a Disc Mill (Buhler DLFU, Buhler AG, CH-Uzwil, Switzerland) using the fine (0.2 mm particle size) setting into barley flour.

Starch Gelatinization (Differential Scanning Calorimetry)

Differential scanning calorimetry was conducted using a modified procedure described previously (Fox et al., 2019). Briefly, 2 mg (± 0.15 mg) of barley flour was weighed into a Tzero aluminum pan (TA Instruments, Delaware, U.S.A.), to which deionized water was added until the total mass was 5 mg (± 0.15 mg). The pan was then dry sealed. The blank used for the testing was an empty pan, which was also dry sealed. Using a DSC-250 differential scanning calorimeter (TA Instruments, Delaware, U.S.A.), the heating regimen started with an initial temperature of 40°C followed by a ramp-up to 75°C with 5°C/min increments. Onset temperature, peak temperature and offset temperature were measured over a peak integration of 55 to 75°C using the Trios v4.2.136612 software program (TA Instruments, Delaware, U.S.A.).

Enthalpy (J/g) and time to peak temperature (min) were also recorded but not analyzed in this study.

Grain weight and size

Fifty grains were taken from a representative sample. The grains were firstly weighed using a weighing scale with accuracy of 0.001 g. The same grain sample was then measured using a vernier caliper, and length and breadth measurements were recorded in mm. This procedure was repeated for all grain samples in duplicate. Plump and thin grains (%) were measured using the industry standard method (American Society of Brewing Chemists, 2012). One hundred grams (± 0.1 g) of sample was passed through four consecutive sieves (2.78 mm [$\frac{7}{64}$ inch], 2.38 mm [$\frac{6}{64}$ inch], 2.18 mm [$\frac{5.5}{64}$ inch] and 1.98 mm [$\frac{5}{64}$ inch]) using a sieve shaker (Sortimat Sample Grader K4, Pfeuffer, Kitzingen, Germany) for 3 min (± 10 s). The sample collected in each sieve was weighed, and the percentages of sample from the 2.78 mm and 2.38 mm sieves were recorded as plump and thin (%) respectively.

Grain protein content

GPC (%) was calculated from total N (using multiplier of 6.25) measured using the near infrared reflectance (NIR) grain analyzer using the industry standard method (American Society of Brewing Chemists, 1984; Nelsen and Lundy, 2020). The results were validated with similar NIR methods by the USDA Malting Quality Lab (Madison, WI).

Grain yield

Grain yield was estimated in kg ha^{-1} from each harvest as described previously, using machine (Wintersteiger Classic) harvested grain, which was cleaned, de-awned, and corrected to 12% moisture content for final yield determination.

Statistical Analysis

Finlay-Wilkinson (FW) regression

A Finlay-Wilkinson regression (Finlay and Wilkinson, 1963) was performed using location-year means and genotype as covariates in a linear model (Eq. 1). The trends were contrasted to determine if they were significantly different (95% confidence interval) from the average using estimated marginal means. The model was fitted in in R 4.2.1 (R Core Team, 2020) and R Studio version 2022.07.1 build 485 (RStudio Team, 2022) using the *FW* (Lian and de Los Campos, 2015) and *emmeans* package (Searle et al., 1980).

$$yield \sim LY\ yield + G + LY\ yield : G \ldots\ldots\ldots\ldots\ldots Eq.1$$

where LY yield is the average yield of each location-year combination.

Modeling

For the first experiment, the linear mixed effects model was run using the *lme4* package (Bates et al., 2015). The dataset including data from 12 genotypes, eight locations and three seasons (2017-18, 2018-19 and 2020-21) was used for linear mixed-effects modeling. The variance components were estimated using a linear model (Eq. 2)

$$Z \approx G + L + Y + GL + GY + LY \ldots\ldots\ldots\ldots\ldots Eq.2$$

where Z is the response trait; GL is the genotype x location interaction, GY is the genotype x year interaction and LY is the location x year interaction. All predictor variables were inputted as random variables with random intercepts and fixed slopes.

For the second experiment, the 2016-17 season which included three locations was used. Replicate plots in each location were trialed with added N levels ranging from 0 to 150 kg ha^{-1}

(0, 20, 60, 90, 120 and 150 kg ha^{-1}). These samples came from a previously described study that examined the effects of the timing of N addition on grain yield (Nelsen and Lundy, 2020). For this study, we utilized data from plots with the most realistic added N levels that are used by farmers in this region (20 to 120 kg ha^{-1}). This dataset was used to assess the effects of added N, and was excluded from the other analyses due to the management-focused experimental design and small number of genotypes (n=2). The variance components were estimated using a linear model (Eq. 3)

$$Z \approx N_1 + N_2 + G + L \quad \ldots\ldots\ldots\ldots\ldots Eq.3$$

where Z is the response trait, N_1 is the N fertilizer application pre-planting and N_2 is the N fertilizer application at tillering. G and L were the effects corresponding to genotype and location. All predictor variables were inputted as random variables with random intercepts and fixed slopes.

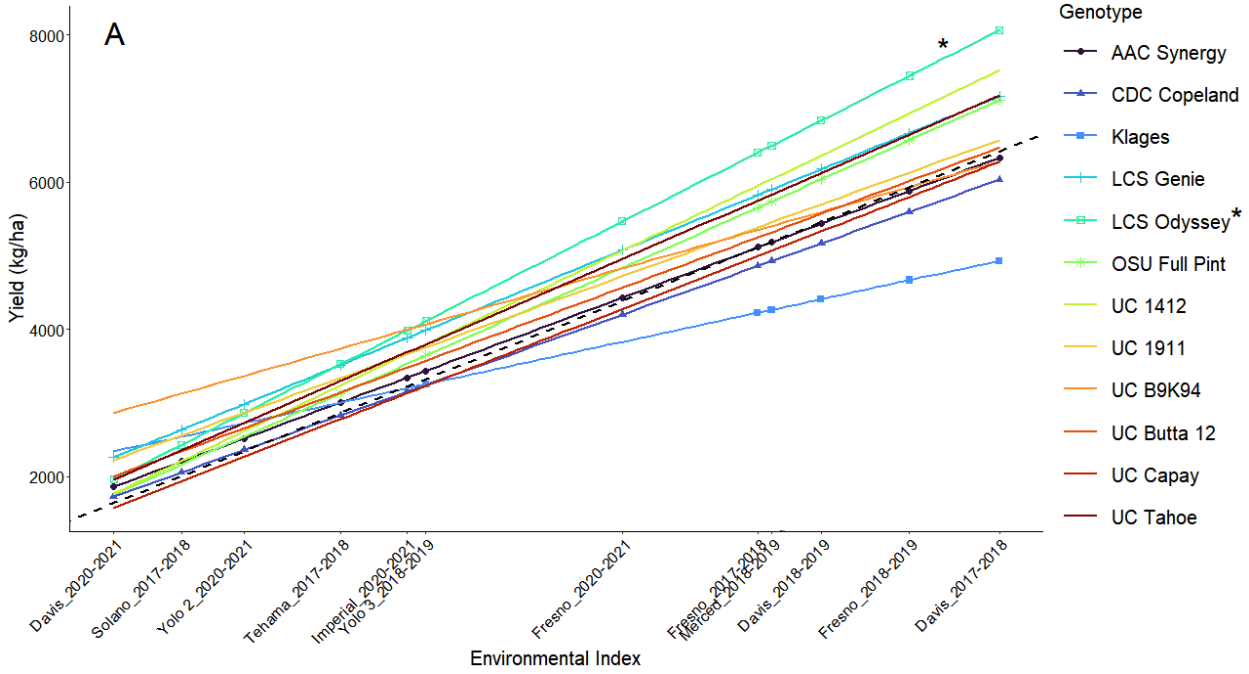
Data Visualization

Data visualization and statistical significance testing were performed using the *tidyverse* (Wickham et al, 2019), *car* (Fox and Weisberg, 2019), *ggplot2* (Wickham, 2016), *corrplot* (Wei and Simko, 2021), *agricolae* (de Mendiburu and Yaseen, 2020), *datasets* (R Core Team, 2020) and *reshape2* (Wickham, 2007) packages. Principal component analysis (PCA) was performed on the full dataset using the *prcomp* function with corresponding biplots developed using the *factoextra* (Kassambara and Mundt, 2020) and *ggbiplot* (Wickham, 2016) packages. Figure S1 was created and modified using Mapline (https://mapline.com). The data and scripts underlying this study are available as supplemental material.

RESULTS AND DISCUSSION

Location overview and genotype adaptability to the California region

The location, coordinate, weather and management information are summarized in Table S1. For the samples in this experiment, the average yields ranged from 1,996 kg ha^{-1} in Davis (2020-21) to 6,646 kg ha^{-1} in Davis (2017-18), and GPC ranged 7.9% in Imperial Valley (2020-21) to 15.1% in Davis (2020-21). Yolo region 3 was an organic site with average yield and GPC of 3,522 kg ha^{-1} and 8.4%, respectively. The present study on malting barley included the 2020-21 season which was reported to be the warmest and driest season in the past century (California Department of Water Resources, 2021).



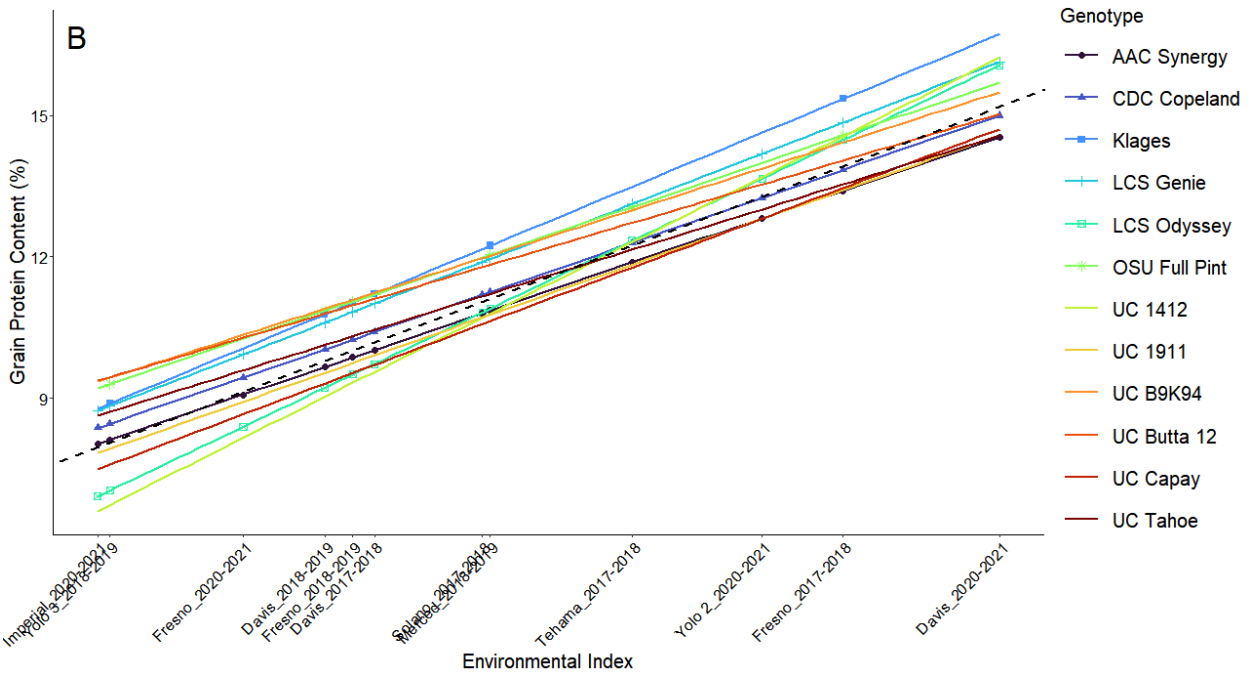


Figure 1. Finlay-Wilkinson regression of the 12 genotypes tested across eight locations and three years for stability of A) yield (kg ha^{-1}); B) GPC (%). Dotted line represents reference slope of 1. * represents a significant difference from reference slope ($p<0.05$).

Yield (kg ha^{-1}) and GPC (%) stability of these genotypes was assessed using Finlay-Wilkinson regression (Figure 1; (Lian and de Los Campos, 2015)). Stability was defined based on the slope of the regression line relative to the reference slope equal to one, with genotypes of higher stability having slopes greater than one and genotypes of lower stability having slopes less than one. All slope values are reported in Table S2.

In terms of yield (Figure 1A), LCS Odyssey (Limagrain Cereal Seeds, 2022) had a significantly higher stability ($p = 0.03$) than all other genotypes for the Californian region. Other genotypes with higher stabilities (slopes not significant) were LCS Genie (Limagrain Cereal Seeds, 2022)), OSU Full Pint, UC Tahoe (Hegarty et al. 2018), UC 1412, and UC Capay (del

Blanco et al., 2022). Genotypes with relatively lower stabilities were AAC Synergy (Legge et al., 2014), CDC Copeland (Canadian Food Inspection Agency, 2007), Klages (Wesenberg et al., 1974), UC 1911, UC B9K94, and UC Butta 12 (Gallagher et al., 2020). AAC Synergy, CDC Copeland and Klages were developed in other production regions, while UC 1911, UC B9K94, and UC Butta 12 were developed for California. It is possible that these genotypes with relatively lower slopes and higher intercepts could be early maturing or genotypes that have been developed for niche and/or low yielding environments. For example, Klages is not recommended for low rainfall regions, or water-limited cropping (Wesenberg et al., 1974).

In terms of GPC (Figure 1B), the genotypes with highest adaptability were Klages, LCS Genie, LCS Odyssey UC 1412 and UC Capay. In summary, all genotypes were relatively stable in GPC, with no significant differences observed in slopes.

Effects of G, L, Y, and their interactions on malting barley productivity and quality

Five traits—yield (kg ha^{-1}), GPC (%), individual-grain weight and grain size (plump and thins)—were examined in a linear mixed effect modeling framework. Genotype, location and year-wise averages are reported in Table S2. The samples from 2020-21 season had relatively smaller grain size and higher onset starch GTs (Table S2).

The percentage of variance explained by the main and interaction effects of G, L, and Y are shown in Table 1. For yield and GPC, the L:Y interaction accounted for 58% and 72% of the variance, respectively. The largest variance in percentage of plump and thin grains was explained by Y and G. The linear model that was fit for individual-grain weight had a large extent of residual variance, potentially indicating that this trait is more dependent on specific management factors that were not explicitly tested in this model.

Table 1. Main and interaction effects of genotype (G), location (L), and year (Y) on traits of relevance to malting barley productivity and quality using linear mixed effects models. 'Res' refers to residuals.

Yield (kg ha^{-1})				Protein content (%)			
	% Explained	Variance	Std. dev.		% Explained	Variance	Std. dev.
G:L	0	0	0	G:L	3	0.203	0.451
G:Y	1	43396	208	G:Y	3	0.193	0.439
L:Y	58	1991726	1411	L:Y	72	4.901	2.214
G	4	144907	381	G	0	0.023	0.152
L	5	157733	397	L	0	0.000	0.000
Y	14	495562	704	Y	0	0.000	0.000
Res	18	623619	790	Res	22	1.469	1.212
Plump (>2.78 mm) %				**Thin (2.38-2.78 mm) %**			
	% Explained	Variance	Std. dev.		% Explained	Variance	Std. dev.
G:L	0	0.000	0.000	G:L	0	0.000	0.000
G:Y	10	63.910	7.994	G:Y	4	14.720	3.837
L:Y	4	25.930	16.100	L:Y	6	24.390	4.938
G	18	114.500	10.700	G	8	34.220	5.850
L	0	0.000	0.001	L	6	25.850	5.084
Y	30	192.700	13.880	Y	30	126.050	11.227
Res	38	244.000	15.620	Res	46	188.730	13.738
Individual-grain weight (mg)							
	% Explained	Variance	Std. dev.				

G:L	1	0.331	0.575				
G:Y	0	0.000	0.000				
L:Y	0	0.000	0.000				
G	0	0.000	0.000				
L	22	12.180	3.491				
Y	0	0.000	0.000				
Res	77	42.120	6.490				

These results (Table 1) are in line with a previous genotype by environment study conducted in Ethiopia (Bantayehu, 2013), where location explained the largest variance in grain quality traits. Interestingly, in studies where the malt quality was assessed as opposed to grain quality (like in the present study), the contribution of variance coming from G was larger than L and/or Y (Nielsen and Munck, 2003; Laidig et al., 2017). This highlights the need for a deeper understanding of how grain quality traits correlate with malt quality traits (e.g., total starch extract %, total β-glucan content). Furthermore, a more in-depth characterization of grain and malt quality parameters in a larger number of genotypes could also be worthwhile to inform selection in earlier stages of the breeding process. Enabling such characterizations at a greater scale and/or with higher throughput could have value to the industry and research community.

Effects of G, L, Y and their interactions on starch GT

The main and interaction effects of G, L and Y on starch GT are shown in Table 2. For onset GT, the largest percentage of variance was explained by Y, followed by G. For peak and offset GT, the largest percentage of variance was explained by G and the L:Y interaction term, respectively. In addition to the onset, peak and offset GT, it is also important to consider the GT

temperature range using the difference between onset and peak, and peak and offset GT. A broader GT range will result in a wider DSC curve, which has been attributed to the presence of more type A starch granules that have been packed heterogeneously (Vasanthan and Bhatty, 1996; Suh et al., 2004). Similar to individual-grain weight, the residual variance of the difference between onset and peak GT was large, suggesting that there could be variance associated with specific management factors that were not explicitly tested in this model.

Table 2. Main and interaction effects of genotype (G), location (L), and year (Y) on starch GT using linear mixed effects models. Units are °C for all of the traits presented in this table. 'Res' refers to residuals.

Onset GT				**Peak GT**			
	% Explained	**Variance**	**Std. dev.**		**% Explained**	**Variance**	**Std. dev.**
G:L	6	0.110	0.332	G:L	0	0.000	0.000
G:Y	9	0.168	0.410	G:Y	7	0.124	0.352
L:Y	12	0.233	0.483	L:Y	25	0.456	0.676
G	23	0.456	0.675	G	36	0.658	0.811
L	1	0.015	0.121	L	0	0.000	0.000
Y	26	0.515	0.718	Y	4	0.083	0.288
Res	24	0.473	0.688	Res	29	0.528	0.727
Offset GT				**Difference between onset and peak GT**			
	% Explained	**Variance**	**Std. dev.**		**% Explained**	**Variance**	**Std. dev.**
G:L	2	0.034	0.185	G:L	7	0.044	0.209
G:Y	5	0.077	0.278	G:Y	9	0.057	0.239
L:Y	30	0.454	0.674	L:Y	1	0.004	0.062

G	23	0.348	0.590	G	0	0.000	0.000
L	0	0.000	0.000	L	6	0.039	0.199
Y	0	0.000	0.000	Y	7	0.043	0.207
Res	39	0.595	0.772	Res	70	0.438	0.661
Difference between peak and offset GT							
	% Explained	**Variance**	**Std. dev.**				
G:L	0	0.000	0.000				
G:Y	0	0.000	0.000				
L:Y	33	0.715	0.267				
G	2	0.046	0.215				
L	6	0.131	0.362				
Y	38	0.821	0.906				
Res	20	0.427	0.653				

The difference between the peak and offset GT was substantially explained by Y and L:Y (Table 2). It is possible these effects were mediated by the amylose (A) to amylopectin (AP) ratio, and the percentage of small granules present in the grain. Amylose (A) and amylopectin (AP) ratios directly impact GT in malting barley, and it has been found that a higher A:AP ratio can trigger higher GT (e.g., higher peak and offset GT; Källman et al. 2015). It was previously reported that a higher A content (%) may cause it to entangle and/or co-crystallize with AP, thereby limiting starch swelling and subsequent hydrolysis (Tester and Morrison, 1990). This could result in an increased starch GT. Further examination of these trends in a wider sample set coming from varied G, L and/or Y is needed for this assessment. High GTs have also been associated with an increased percentage of smaller starch granules in the barley endosperm

(Langenaeken et al., 2019). These smaller starch granules are often developed due to changes in starch biosynthesis during grain development that are triggered by drought (Gous et al., 2015). Hence, a large extent of variance in the difference between peak and offset GT being explained by Y could prove to be problematic for the malting and brewing industries.

Effect of nitrogen fertilization on malting barley productivity and quality

In order to study the effect of added N on malting barley quality and starch gelatinization, dataset from the 2016-17 season consisting of three locations, two genotypes and multiple N treatments applied prior to planting or at tillering growth stage was leveraged. For the Davis, Rio Vista and Tulelake sites in this experiment, the average grain yield/GPC were 4478 kg ha^{-1}/9.8%, 3160 kg ha^{-1}/12.6% and 7178 kg ha^{-1}/12.3%, respectively. The results of linear mixed-effects modeling in this dataset indicated that L and G accounted for the largest variance in all starch GT traits, except the difference between onset and peak GT (Table 3). Added N levels at tillering accounted for 18% of the variance in this trait. However, pre-plant added N levels did not account for any variance in GT traits. This could be because of the tillering stage being closer to growth stages related to heading and grain-fill when the starch granules in the grain are developed. L accounted for the largest variance in percentage of plump and thin grains.

Table 3. Effect of added nitrogen levels pre-planting in kg ha^{-1} (N_1), at tillering in kg ha^{-1} (N_2), location (L) and genotype (G) on malting barley productivity and quality using linear mixed effects models. Units are °C for all of the GT-related traits presented in this table. 'Res' refers to residuals.

Onset GT				Peak GT			
	% Explained	Variance	Std. dev.		% Explained	Variance	Std. dev.

N_1	0	0.000	0.000	N_1	0	0.000	0.000
N_2	0	0.000	0.000	N_2	0	0.000	0.000
L	53	0.651	0.807	L	65	1.023	1.011
G	17	0.210	0.458	G	3	0.049	0.230
Res	30	0.374	0.611	Res	32	0.499	0.707
Offset GT				**Difference between onset and peak GT**			
	% Explained	**Variance**	**Std. dev.**		**% Explained**	**Variance**	**Std. dev.**
N_1	0	0.000	0.000	N_1	0	0.000	0.000
N_2	0	0.000	0.000	N_2	18	0.058	0.241
L	49	2.266	1.505	L	27	0.084	0.290
G	37	1.713	1.309	G	9	0.028	0.168
Res	15	0.691	0.831	Res	46	0.144	0.380
Difference between peak and offset GT				**Yield (kg ha^{-1})**			
	% Explained	**Variance**	**Std. dev.**		**% Explained**	**Variance**	**Std. dev.**
N_1	0	0.000	0.000	N_1	0	0	0
N_2	3	0.085	0.292	N_2	0	0	0
L	11	0.331	0.575	L	78	6348000	2520
G	79	2.449	1.565	G	16	1348000	1161
Res	7	0.226	0.475	Res	6	490500	700
Plump (>2.78 mm) %				**GPC (%)**			
	% Explained	**Variance**	**Std. dev.**		**% Explained**	**Variance**	**Std. dev.**
N_1	0	0.000	0.000	N_1	2	0.078	0.279
N_2	0	0.000	0.000	N_2	5	0.187	0.432
L	72	202.650	14.236	L	47	1.745	1.321

G	10	28.190	5.309	G	44	1.643	1.282
Res	17	48.680	6.977	Res	2	0.078	0.279
Thin (2.38-2.78 mm) %				**Individual-grain weight (mg)**			
	% Explained	**Variance**	**Std. dev.**		**% Explained**	**Variance**	**Std. dev.**
N_1	0	0.000	0.000	N_1	0	0.000	0.000
N_2	0	0.000	0.000	N_2	0	0.000	0.000
L	70	123.400	11.110	L	11	11.248	3.354
G	13	23.480	4.845	G	85	88.692	9.418
Res	16	28.540	5.342	Res	4	3.838	1.959

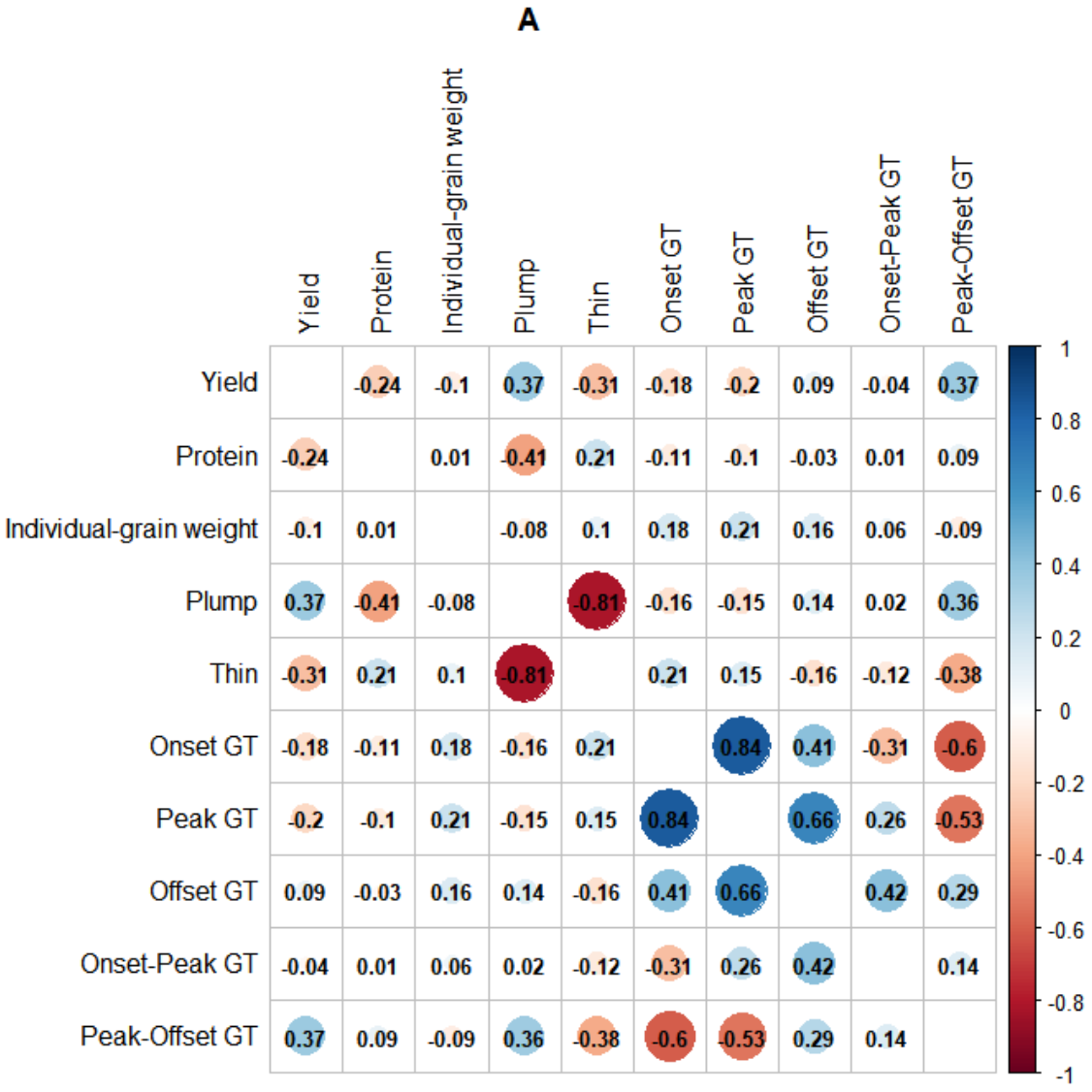
Interestingly, the added N levels were not found to account for variance in grain yield. In terms of GPC, L and G once again accounted for the largest variance. Out of the variance in GPC coming from added N levels, 5% was explained by application at tillering and 2% was explained by application pre-planting. A global meta-analysis on durum wheat reported that later season N application increased protein levels but consistently did not contribute to grain yield (Giordano et al., 2023). Previous findings in California also have reported that late season application of N can influence GPC in malting barley (Nelsen and Lundy, 2020) and bread wheat (Wuest and Cassman, 1992). Top dress N application at early heading was found to be more influential in increasing GPC, compared to early season addition in malting barley, from a recent study in the Pacific Northwest US (Halstead et al., 2022). Our findings are consistent with the above observations.

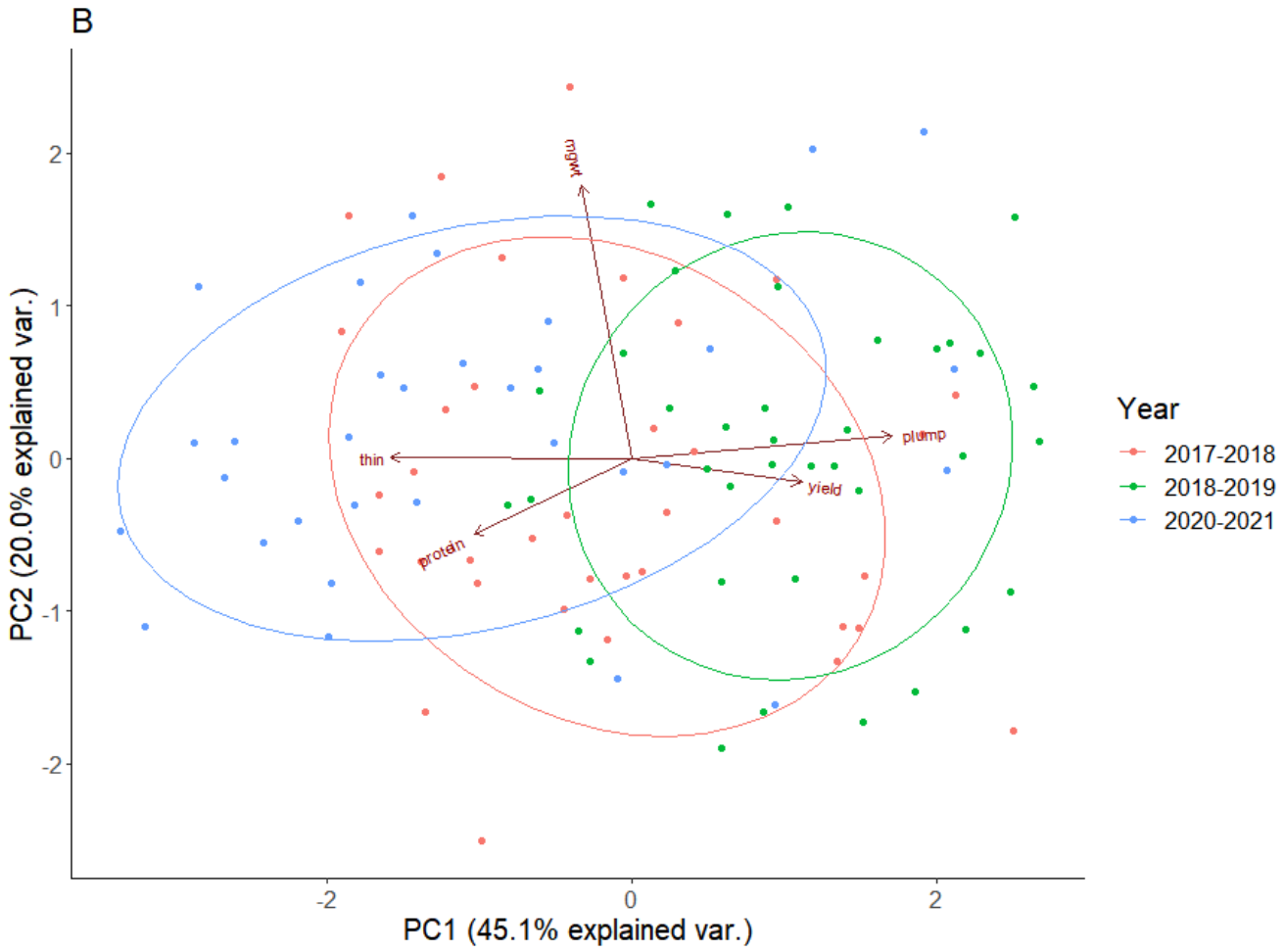
Furthermore, N uptake by the plant is predicated on soil water availability (Prystupa et al., 2018). Precipitation totals have previously been found to impact the effects of N fertilizer

applications in winter wheat (Zebarth and Sheard, 1992), with low and high precipitation levels influencing the effects of N timing and rate on grain yields. In our study, soil water availability to the crops at the different locations varied dramatically. At each site, N fertilizer was applied prior to/in coincidence with precipitation and/or irrigation to ensure N incorporation into the soil during the fertilization event itself. However, this does not imply that water supply was sufficient to meet crop demand for the remainder of the season. It is likely that water-limitation later in the season impacted seasonal N uptake patterns and prevented efficient/complete seasonal N uptake at some of the locations. Therefore, one hypothesis is that the applied N levels did not contribute to considerable variance in yield and GPC in the current study due to large differences in soil water availability among locations and potential water limitations impacting N assimilation. While our results indicate that G and L contribute to a greater degree of variance in comparison to N fertilizer application, this dataset was only from one year (2016-17). A dataset with a higher resolution (including samples from multiple years) might be required to study interaction effects between location and N levels.

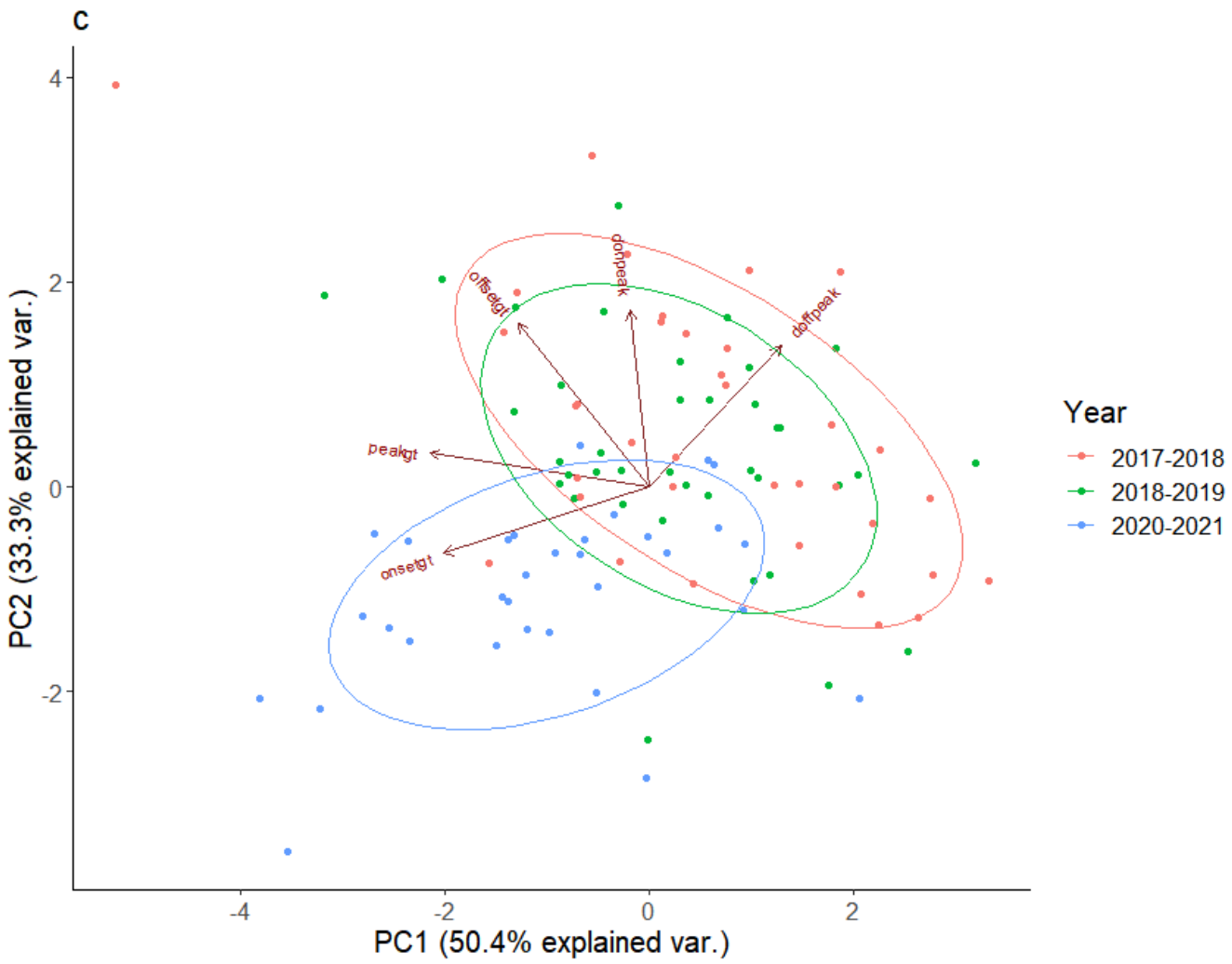
Trait relationships (correlations and principal component analysis)

Pearson correlations were examined between the malting barley productivity and quality traits studied herein (Figure 2A). Correlations discussed here are indicated using colored circles and were statistically significant based on a 95% asymptotic confidence interval using Fisher's Z transform. Yield was positively correlated with plump % (r = 0.37) and the difference between peak and offset GT (0.37), but was negatively correlated with thin % (-0.31) and peak GT (-0.20). GPC was negatively correlated with plump % (-0.41) and yield (-0.24). Peak GT was negatively correlated with yield (-0.24) and plump % (-0.15), but was positively correlated with individual-grain weight (0.18).


A
Yield
Protein
Individual-grain weight
Plump
Thin
Onset GT
Peak GT
Offset GT
Onset-Peak GT
Peak-Offset GT
Yield -0.24 -0.1 0.37 -0.31 -0.18 -0.2 0.09 -0.04 0.37
Protein -0.24 0.01 -0.41 0.21 -0.11 -0.1 -0.03 0.01 0.09
Individual-grain weight -0.1 0.01 -0.08 0.1 0.18 0.21 0.16 0.06 -0.09
Plump 0.37 -0.41 -0.08 -0.81 -0.16 -0.15 0.14 0.02 0.36
Thin -0.31 0.21 0.1 -0.81 0.21 0.15 -0.16 -0.12 -0.38
Onset GT -0.18 -0.11 0.18 -0.16 0.21 0.84 0.41 -0.31 -0.6
Peak GT -0.2 -0.1 0.21 -0.15 0.15 0.84 0.66 0.26 -0.53
Offset GT 0.09 -0.03 0.16 0.14 -0.16 0.41 0.66 0.42 0.29
Onset-Peak GT -0.04 0.01 0.06 0.02 -0.12 -0.31 0.26 0.42 0.14
Peak-Offset GT 0.37 0.09 -0.09 0.36 -0.38 -0.6 -0.53 0.29 0.14
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1


B
PC2 (20.0% explained var.)
PC1 (45.1% explained var.)
2
1
0
-1
-2
-2
0
2
mgmt
thin
plump
yield
protein
Year
2017-2018
2018-2019
2020-2021

363



364

365 Figure 2. A) Pearson correlation coefficient (*r*) matrix between malting barley productivity and
366 quality traits. Colored circles indicate significant correlations based on a 95% asymptotic
367 confidence interval using Fisher's Z transform; Year based biplots for B) productivity and
368 quality traits PCs 1 and 2; C) GT traits PCs 1 and 2. Vectors represent yield in kg ha^{-1} (*yield*),
369 GPC (*protein*), percentage of plump (*plump*) and thin (*thin*) grains, individual-grain weight in
370 mg (*mgwt*), onset GT (*onsetgt*), peak GT (*peakgt*), offset GT (*offsetgt*), difference between onset
371 and peak GT (*donpeak*), and the difference between peak and offset GT (*doffpeak*). Normal

confidence ellipses based on multivariate t-distribution were drawn with 95% confidence intervals for all biplots.

The correlation between yield and GPC is generally accepted to be negative in malting barley (Fox, 2010). However newer studies have shown that potential explanations for this negative correlation could be due to nitrogen availability (Magliano et al., 2014) and/or tiller formation (Hu et al., 2021). The negative correlation between peak GT and percentage of plump grains is expected as it has been previously established that plump grains also contain an overall higher extractable starch content, therefore requiring a higher peak GT to hydrolyze the starch (Andersson et al., 1999; Vahamidis et al., 2022).

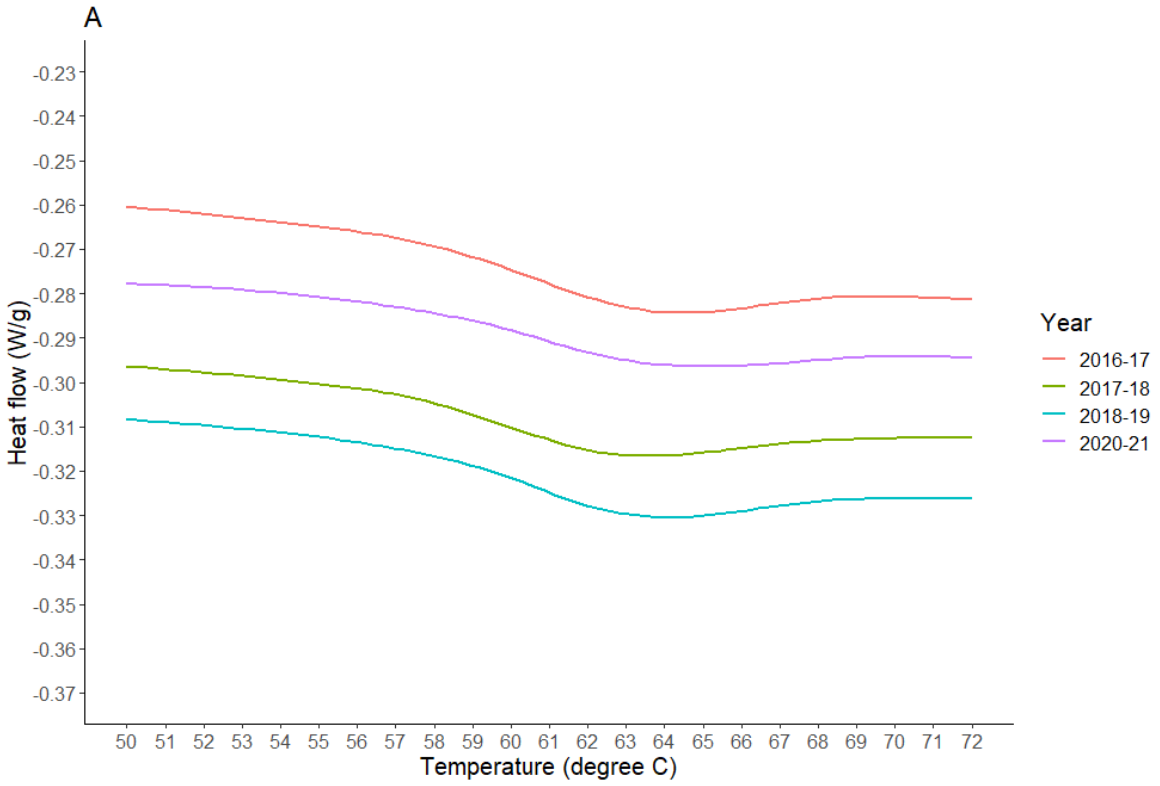
The difference between peak and offset GT showed a strong negative correlation with the onset GT (-0.60), peak GT (-0.53) and thin % (-0.38), but a positive correlation with yield (0.37) and plump % (0.36). This trait (i.e., difference between peak and offset GT) has not been previously reported, and suggests that some alternative endosperm parameter not assessed in this study could be causing a delay in the gelatinization of starch above the peak temperature. One potential explanation to this correlation could be attributed to the proportion of A-type and B-type starch granules within the endosperm (Goering et al., 1973; Vasanthan and Bhatty, 1996). The smaller B-type granules which are developed later in the grain filling process have been previously shown to gelatinize more slowly than A-type granules (Karlsson et al., 1983; Langenaeken et al., 2019). Hence it is possible that the samples with a higher difference between the peak and offset GT assessed in this study could contain a higher proportion of B-type granules than A-type granules. Another possibility could be the variation in hordein (major seed

storage proteins in the endosperm) levels which could impact the accessibility of starch granules to starch-degrading enzymes (Wenwen et al., 2019). Further research on starch granule proportions and hordein content will enable a more comprehensive understanding of this complex relationship between starch GT and grain quality.

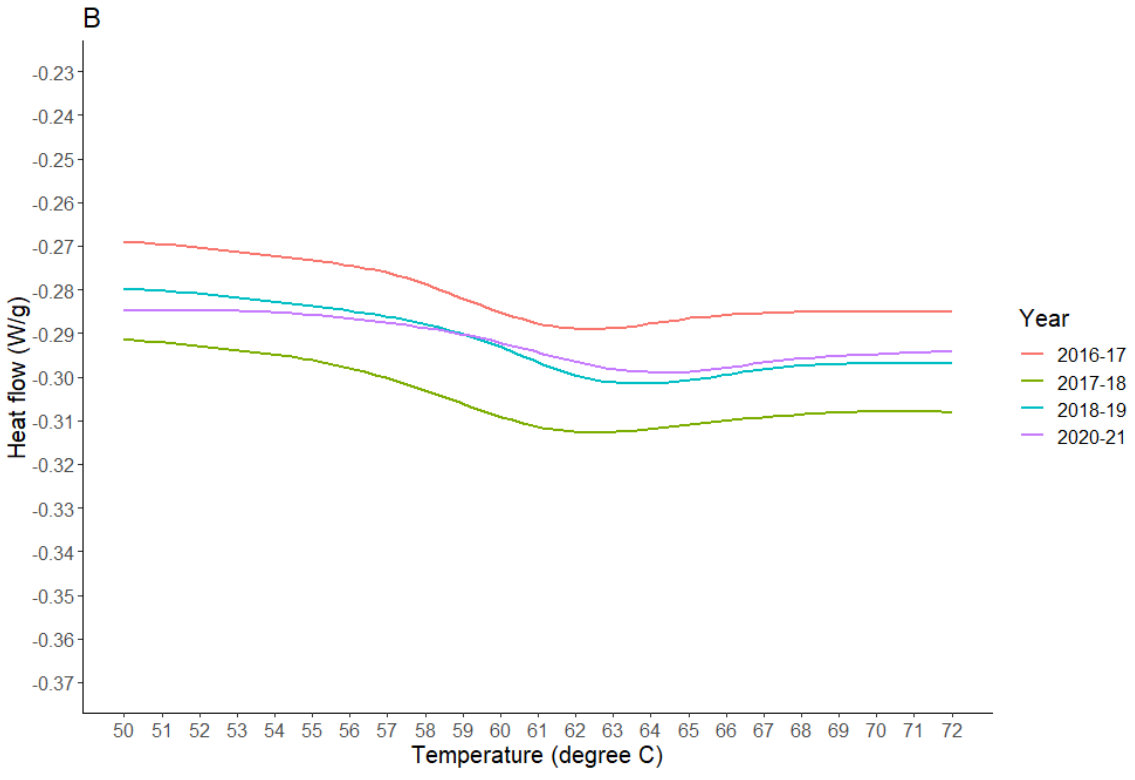
PCA biplots were used to visualize the relationships among traits across three years (Figure 2B and 2C). Normal confidence ellipses based on multivariate t-distribution were drawn with 95% confidence intervals for each year. For productivity and quality traits, the first, second, and third principal components (PCs) explained 45%, 20% and 16.9% of the total variance, respectively. For the starch GT traits, the first and second PCs explained 55.4% and 33.3% of the total variance, respectively. The 2020-21 season samples formed a partially distinct cluster, mainly discriminated by high percentage of thin grains (Figure 2B) and high onset GT (Figure 2C).

Gelatinization profiles (differential scanning calorimetry curves)

The average values of the malting barley productivity and grain quality traits based on year, location and genotype were examined to assess key differences (Table S2). The 2020-21 year had relatively higher average values for onset, peak and offset GT and lower percentage of plump grains. Figure 3 illustrates how the starch gelatinization curves for UC Tahoe and UC Capay differ across the four seasons. On average, the onset GT was higher for the 2020-21 season in comparison to other seasons for both UC Tahoe and UC Capay. This was also in line with the PCA results observed for this season (Figure 2C).


A
Heat flow (W/g)
-0.23
-0.24
-0.25
-0.26
-0.27
-0.28
-0.29
-0.30
-0.31
-0.32
-0.33
-0.34
-0.35
-0.36
-0.37
50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72
Temperature (degree C)
Year
2016-17
2017-18
2018-19
2020-21

415


B
Heat flow (W/g)
-0.23
-0.24
-0.25
-0.26
-0.27
-0.28
-0.29
-0.30
-0.31
-0.32
-0.33
-0.34
-0.35
-0.36
-0.37
50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72
Temperature (degree C)
Year
2016-17
2017-18
2018-19
2020-21

416

Figure 3. Average differential scanning calorimetry curves for A) UC Tahoe grown in 2016-17 (N= 2 locations), 2017-18 (N= 4), 2018-19 (N= 4) and 2020-21 (N= 3); B) UC Capay grown in 2016-17 (N= 1), 2017-18 (N= 4), 2018-19 (N= 4) and 2020-21 (N =3).

The 2020-21 season was characterized by a higher average maximum temperature during crop growth cross the locations tested (Table S1). Moreover, while a few sites in other seasons also experienced drought conditions (i.e., based on crop evapotranspiration in excess of soil water supply during the reproductive growth phase and accompanying observations of drought-related symptoms). Three out of four sites in the 2020-21 season experienced terminal drought stress as also indicated by the water (precipitation and irrigation) levels post heading in Table S3. Hence one possible explanation is that the extreme weather conditions during grain fill could have led to the formation of smaller (i.e., a higher percentage of B-type) starch granules within the endosperm, which subsequently could have led to higher onset, peak and offset GTs. Colder summer temperatures have been shown to lower the peak GT in a barley study in Finland (Myllärinen et al., 1998) compared to climate-typical summers, which is further indicative of an inverse relationship between starch GT and growing season temperature.

CONCLUSION

To conclude, this study was the first assessment of the combined effects of G, L, Y and added N levels on starch gelatinization. It was also the first study to assess malting barley productivity and grain quality for the Californian region. The largest variance in yield, GPC, plump and thin grains, and individual-grain weight were explained by either L, Y or their interaction. We also confirmed that Y and the L x Y interaction term explained the largest

variance in onset and offset starch GT, respectively, but the largest variance in peak GT was explained by G. Added N levels accounted for only 5% variance in GPC, but accounted for 18% of variance in the difference between onset and peak GT. The effect of added N levels was minimal for all other traits. Finally, PCA of the same dataset used for the FW regression shows that the 2020-21 season formed partially distinct clusters, segregated by a high percentage of thin grains and high onset GT. These findings illustrate the critical role of G, L and Y in determining malting barley productivity and grain quality in California.

ACKNOWLEDGMENTS

To be added post acceptance.

SUPPLEMENTAL MATERIAL

The supplemental material includes tables detailing coordinates, climatic and management information of the locations (Table S1), average trait values by year, location and genotype (Table S2) and pre and post heading water levels for locations per year (Table S3). It also includes a map of California where the field trials were conducted (Figure S1). Other figures (Figure S2 and S3) are scree plots corresponding to the biplots in the main manuscript, and biplots corresponding to PCs 1 and 3; and 2 and 3. PCA biplots based on location are shared in Figure S4. It also includes the R script and associated data files that were used for data analysis and visualization.

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