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

Maany Ramanan[1], Taylor Nelsen[1], Mark Lundy[1], Christine Diepenbrock[1], and Glen Fox[1]

[1]University of California, Davis

December 7, 2022

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.

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

Maany Ramanan[a], Taylor Nelsen[b], Mark Lundy[b,c], Christine Diepenbrock[b*], Glen Patrick Fox[a*]

[a] Department of Food Science & Technology, University of California, Davis, USA
[b] Department of Plant Sciences, University of California, Davis, USA
[c] University of California Cooperative Extension, University of California, Davis, USA
* For correspondence: chdiepenbrock@ucdavis.edu, gpfox@ucdavis.edu.

1   **Core Ideas**

2   1. The Location x Year interaction explained the majority of the variance for GPC and yield.

3   2. Added nitrogen fertilizer levels at tillering accounted for 5% of the variance in Grain Protein

4   Content.

5   3. Y, Genotype and L x Y explained the largest variance in onset, peak and offset GT,

6   respectively.

7   4. The 2020-21 samples formed partially distinct clusters, segregated by high thins and onset GT.

8   5. Plumper grains had a lower onset GT but a larger difference between peak and offset GT.

9

10

11   ASBC, American Society of Brewing Chemists; GT, Gelatinization temperature; G, Genotype;

12   GPC, Grain Protein Content; LME, Linear Mixed Effects; L, Location; N, Nitrogen; UC,

13   University of California; Y, Year

14   **ABSTRACT**

15   Malting barley productivity and grain quality are of critical importance to the malting and

16   brewing industry. In this study, we analyzed two experiments: a multi-environment variety trial

17   and a nitrogen management trial. In the first experiment, we analyzed 12 malting barley

18   genotypes across eight locations in California and three years (2017-18, 2018-19 and 2020-21).

19   The effects of genotype (G), location (L), year (Y) and their interactions were assessed on grain

20   yield (kg ha^{-1}), grain protein content (GPC; %), individual-grain weight, grain size (plump and

21   thin; %), onset gelatinization temperature (GT), peak GT, offset GT, difference between onset

22 and peak GT and difference between peak and offset GT. L, Y and their interaction explained the

23 largest variance for all traits except peak GT and difference between onset and peak GT, for

24 which G explained the largest variance. The 2020-21 samples formed partially distinct clusters in

25 principal component analysis, mainly discriminated by high percentage of thin grains and high

26 onset GT. In the second experiment, we analyzed a dataset with two genotypes across three

27 locations (with varying nitrogen fertilizer levels) from the 2016-17 season to assess the effect of

28 added nitrogen on the same traits. Added nitrogen at tillering explained 18% of variance in the

29 difference between onset and peak GT, and 5% of the variance in GPC, but was minimal for all

30 other traits, with the largest variance explained by location and genotype. These findings

31 illustrate the key roles of G, L and Y in determining malting barley productivity and quality.

32   **INTRODUCTION**

33   Grain of malting barley is typically composed of 50 to 68% starch (Newman and

34 Newman, 1992; You and Izydorczyk, 2007; Patindol et al., 2012), of which 70 to 80% is

35 typically amylopectin and the remaining 20 to 30% is amylose (Greenwood and Thomson, 1959;

36 Vasanthan and Bhatty, 1996; Izydorczyk et al., 2001; Källman et al., 2015). Amylose consists of

37 linear chains of glucose units that are linked by α (1-4) glycosidic bonds. Amylopectin is a

38 branched polymer composed of glucose units linked by both α (1-4) and α (1-6) glycosidic

39 bonds.

40   Barley starch composition plays an important role during the mashing stage of the

41 brewing process (Briggs, 1998). Mashing is carried out as part of the brewing process to extract

42 sugars from the grain into the wort (i.e., infusion of malt), which is later fermented by yeasts to

43 produce beer. During mashing, starch is hydrolyzed to fermentable sugars such as maltose and

44 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

68   California, malting barley growing regions are primarily located in the Sacramento and San

69   Joaquin Valleys and south-Central Coast. In 2021, approximately 20% of malting barley

70   produced in the state of California was grown in the Tulelake basin (Siskiyou County) as a

71   rotation crop with potato, onion and alfalfa (Lindblad, 2022). In the Sacramento and San Joaquin

72   Valleys and the Southern desert region, feed barley is grown predominantly as a rotation crop.

73   These environments form part of the Central Valley and Imperial Valley of California with

74   Mediterranean, semi-arid, and arid desert type climate profiles. Since malting barley is often

75   grown as a rotation crop in a wide range of conditions (Kanter et al., 2021), it is critical to

76   understand the impact of location on malting barley grain quality. Mediterranean climates are

77   known for their temporal variability, driven by hot and dry summers and rainy winter spells with

78   more frequent weather extremes (Nelsen and Lundy, 2020; Hochman et al., 2021). Despite these

79   variable conditions, field crop acreage in California is projected to increase with increased

80   advocacy of water-limited winter cropping systems in light of the recent Sustainable

81   Groundwater Management Act (Peterson et al., 2022). However, the contributions of warming

82   and increased incidents of drought to grain quality are still unknown.

83   The effects of genotype and environment (locations and/or years) have been previously

84   studied for assessing malting barley quality in multiple barley production regions worldwide

85   (Nielsen and Munck, 2003; Bantayehu, 2013; Przulj et al., 2014; Laidig et al., 2017) and in the

86   US (Zhou et al., 2020; Choi et al., 2020). These studies have found the interaction between

87   genotype and environment to play an important role in malting barley quality. However, the

88   traits measured by these studies either were agronomic traits or were grain quality traits

89   stipulated by the American Malting Barley Association. In order to understand the impact of G,

90   L and Y on downstream processing outcomes during brewing, it is important to consider more

91   in-depth traits relating to starch gelatinization.

92   Studies have also found substantial variation in starch GT between multiple genotypes of

93   malting barley (Gujral et al., 2013; Jaiswal et al., 2014; Pycia et al., 2015). Other studies have

94   found that location (Fox et al., 2001), year (Przulj et al., 2014) and environmental factors such as

95   drought stress (Gous et al., 2015) also affect starch GTs. However, these studies were not

96   designed to include multiple locations, years and genotypes. In all cases, up to two out of the

97   three were varied, while keeping the third variable constant. A multi-environment study has been

98   conducted in California to assess yield performance in wheat (George and Lundy, 2019), but

99   malting barley and quality traits have not received the same level of attention in this region to

100   date. A major U.S genome-wide association study identified markers exhibiting significant

101   associations with multiple malting barley quality traits; these instances of potential pleiotropy

102   could make genomics-assisted selection for a full suite of quality traits challenging for breeders

103   (Mohammadi et al., 2015).

104   This study aims to elucidate the complexity of maintaining malting barley productivity

105   and quality in the context of inter-annual temperature and precipitation variability. In the two

106   experiments analyzed herein, we assessed the following traits: grain yield (kg ha^{-1}), grain protein

107   content (GPC; %), individual-grain weight, grain size (percentage of plump and thin grains),

108   onset GT, peak GT, offset GT, difference between onset and peak GT, and difference between

109   peak and offset GT. The first experiment examined whether genotype (G), location (L) and/or

110   year (Y) play a more important role in affecting the aforementioned traits using samples from a

111   multi-environment variety trial. Furthermore, correlations between these malting barley

112   productivity and quality traits were also examined, with an aim to generate hypotheses at the

113   molecular/compositional level. The second experiment was focused on assessing the impact of

114   added nitrogen (N) levels on the same traits. Variation in barley productivity and quality traits

115   arising from G, L and/or Y combinations is leveraged herein to understand relationships among

116   traits affecting end usability for maltsters and brewers.

MATERIALS AND METHODS

Barley

Multi-environment variety trial

120   The samples used were collected from variety trials conducted in 2017-2018, 2018-2019 and

121   2020-2021, by the University of California Small Grains Research team. These trials were

122   planted in a randomized complete block design with four replications across all trial locations

123   (UC-ANR, 2020; Nelsen and Lundy, 2020; Nelsen et al., 2021a). Grain from one out of the four

124   replicates was used for further analysis. Twelve genotypes (9 varieties and 3 experimental lines)

125   of two-row spring malting barley that were developed in the U.S. were included in this study,

126   which were grown in eight field sites within California (Table S1; Figure S1). These plots were

127   planted in the fall season (Table S1), in line with common agronomic practice in California

128   (Jackson et al., 2006). These field trials were conducted in different areas of the state where

129   malting barley is typically grown, with varying management practices based on the initial soil

130   moisture and N levels at each location/year. Statewide yield statistics were obtained using the

131   *tidyUSDA* package (Lindblad, 2022) in R 4.2.1 (R Core Team, 2020). The precipitation and

132   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 ($\pm$ 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 ($\pm$ 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.).

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

156   study.

Grain weight and size

158   Fifty grains were taken from a representative sample. The grains were firstly weighed

159   using a weighing scale with accuracy of 0.001 g. The same grain sample was then measured

160   using a vernier caliper, and length and breadth measurements were recorded in mm. This

161   procedure was repeated for all grain samples in duplicate. Plump and thin grains (%) were

162   measured using the industry standard method (American Society of Brewing Chemists, 2012).

163   One hundred grams (± 0.1 g) of sample was passed through four consecutive sieves (2.78 mm [

164    $\frac{7}{64}$ 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

165   (Sortimat Sample Grader K4, Pfeuffer, Kitzingen, Germany) for 3 min (± 10 s). The sample

166   collected in each sieve was weighed, and the percentages of sample from the 2.78 mm and 2.38

167   mm sieves were recorded as plump and thin (%) respectively.

Grain protein content

169   GPC (%) was calculated from total N (using multiplier of 6.25) measured using the near

170   infrared reflectance (NIR) grain analyzer using the industry standard method (American Society

171   of Brewing Chemists, 1984; Nelsen and Lundy, 2020). The results were validated with similar

172   NIR methods by the USDA Malting Quality Lab (Madison, WI).

Grain yield

174   Grain yield was estimated in kg ha^{-1} from each harvest as described previously, using

175   machine (Wintersteiger Classic) harvested grain, which was cleaned, de-awned, and corrected to

176   12% moisture content for final yield determination.

Statistical Analysis

178 **Finlay-Wilkinson (FW) regression**

179   A Finlay-Wilkinson regression (Finlay and Wilkinson, 1963) was performed using

180 location-year means and genotype as covariates in a linear model (Eq. 1). The trends were

181 contrasted to determine if they were significantly different (95% confidence interval) from the

182 average using estimated marginal means. The model was fitted in in R 4.2.1 (R Core Team,

183 2020) and R Studio version 2022.07.1 build 485 (RStudio Team, 2022) using the *FW* (Lian and

184 de Los Campos, 2015) and *emmeans* package (Searle et al., 1980).

185
$$yield \sim LY\ yield + G + LY\ yield : G \dots\dots\dots\dots\dots Eq.\,1$$

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

187 **Modeling**

188   For the first experiment, the linear mixed effects model was run using the *lme4* package

189 (Bates et al., 2015). The dataset including data from 12 genotypes, eight locations and three

190 seasons (2017-18, 2018-19 and 2020-21) was used for linear mixed-effects modeling. The

191 variance components were estimated using a linear model (Eq. 2)

192
$$Z \approx G + L + Y + GL + GY + LY \dots\dots\dots\dots\dots Eq.\,2$$

193 where Z is the response trait; GL is the genotype x location interaction, GY is the genotype x

194 year interaction and LY is the location x year interaction. All predictor variables were inputted as

195 random variables with random intercepts and fixed slopes.

196   For the second experiment, the 2016-17 season which included three locations was used.

197 Replicate plots in each location were trialed with added N levels ranging from 0 to 150 kg ha^{-1}

198   (0, 20, 60, 90, 120 and 150 kg ha^{-1}). These samples came from a previously described study that

199   examined the effects of the timing of N addition on grain yield (Nelsen and Lundy, 2020). For

200   this study, we utilized data from plots with the most realistic added N levels that are used by

201   farmers in this region (20 to 120 kg ha^{-1}). This dataset was used to assess the effects of added N,

202   and was excluded from the other analyses due to the management-focused experimental design

203   and small number of genotypes (n=2). The variance components were estimated using a linear

204   model (Eq. 3)

205
$$Z \approx N_1 + N_2 + G + L \dots \dots \dots \dots \dots Eq.\,3$$

206   where Z is the response trait, N_1 is the N fertilizer application pre-planting and N_2 is the N

207   fertilizer application at tillering. G and L were the effects corresponding to genotype and

208   location. All predictor variables were inputted as random variables with random intercepts and

209   fixed slopes.

210   **Data Visualization**

211   Data visualization and statistical significance testing were performed using the *tidyverse*

212   (Wickham et al, 2019)*, car* (Fox and Weisberg, 2019)*, ggplot2* (Wickham, 2016)*, corrplot* (Wei

213   and Simko, 2021)*, agricolae* (de Mendiburu and Yaseen, 2020)*, datasets* (R Core Team, 2020)

214   and *reshape2* (Wickham, 2007) packages. Principal component analysis (PCA) was performed

215   on the full dataset using the *prcomp* function with corresponding biplots developed using the

216   *factoextra* (Kassambara and Mundt, 2020) and *ggbiplot* (Wickham, 2016) packages. Figure S1

217   was created and modified using Mapline (https://mapline.com). The data and scripts underlying

218   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

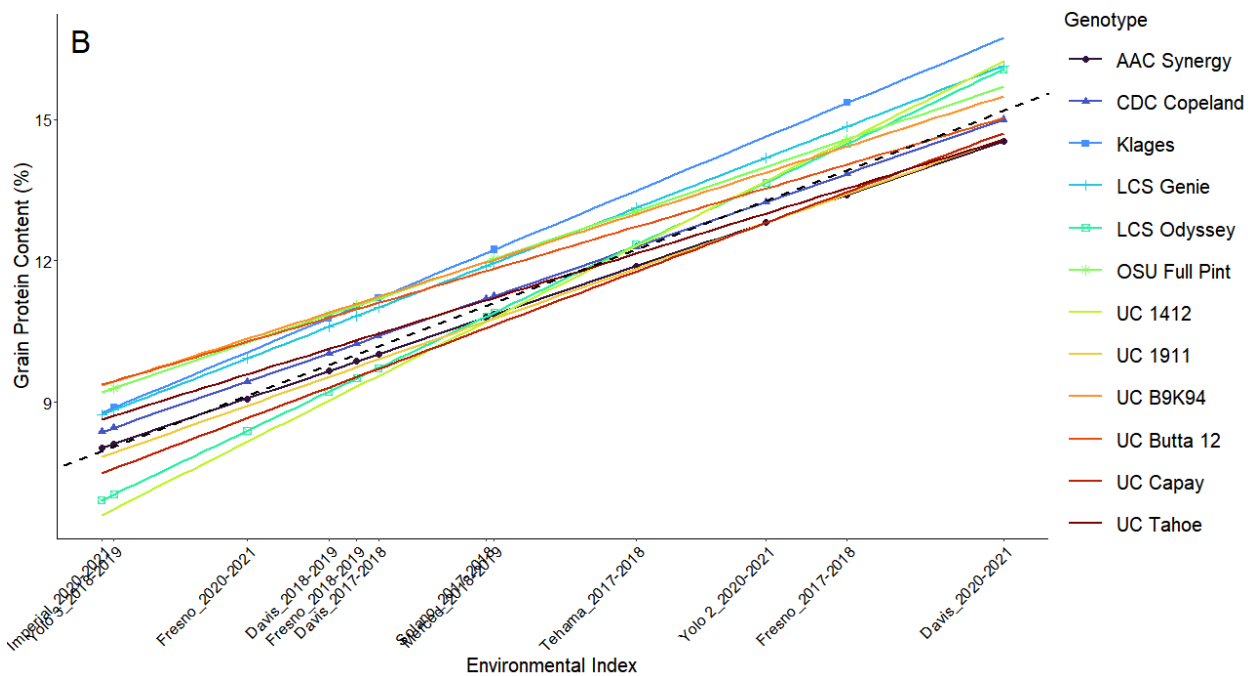Department of Water Resources, 2021).
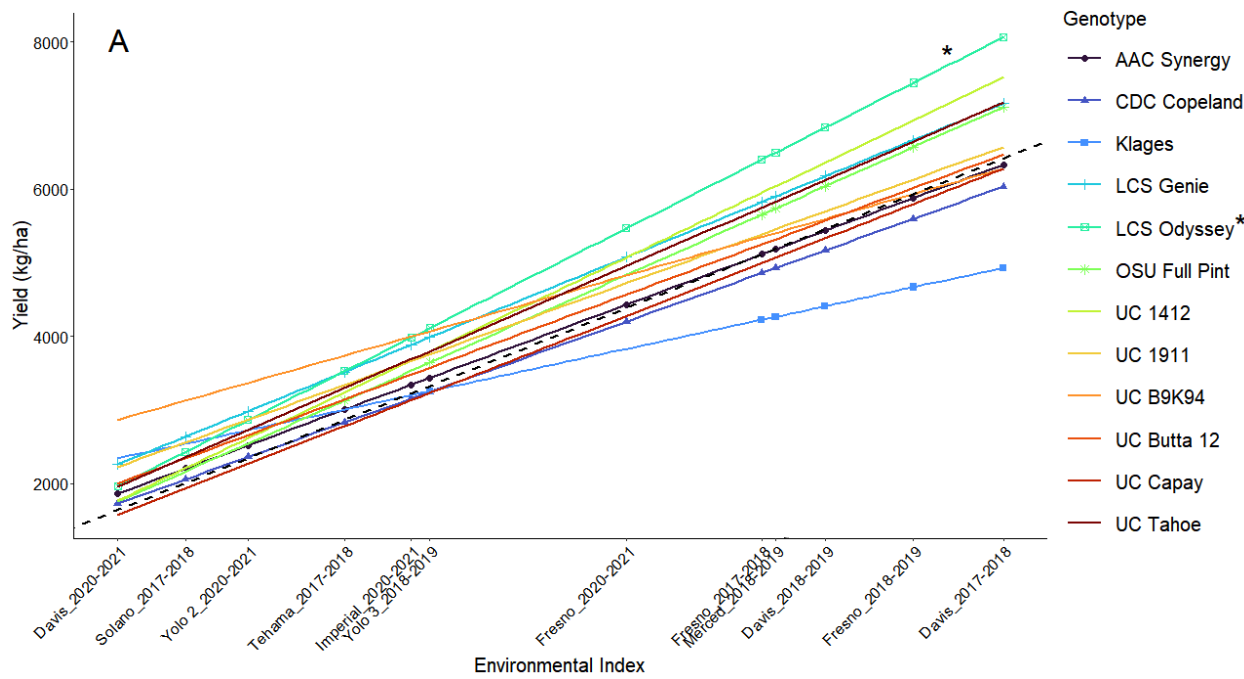
Figure 1. Finlay-Wilkinson regression of the 12 genotypes tested across eight locations and three years for stability of A) yield (kg ha^{-1}); B) GPC (%). Dotted line represents reference slope of 1. * represents a significant difference from reference slope (p<0.05).

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

243　Blanco et al., 2022). Genotypes with relatively lower stabilities were AAC Synergy (Legge et al.,

244　2014), CDC Copeland (Canadian Food Inspection Agency, 2007), Klages (Wesenberg et al.,

245　1974), UC 1911, UC B9K94, and UC Butta 12 (Gallagher et al., 2020). AAC Synergy, CDC

246　Copeland and Klages were developed in other production regions, while UC 1911, UC B9K94,

247　and UC Butta 12 were developed for California. It is possible that these genotypes with relatively

248　lower slopes and higher intercepts could be early maturing or genotypes that have been

249　developed for niche and/or low yielding environments. For example, Klages is not recommended

250　for low rainfall regions, or water-limited cropping (Wesenberg et al., 1974).

251　　In terms of GPC (Figure 1B), the genotypes with highest adaptability were Klages, LCS

252　Genie, LCS Odyssey UC 1412 and UC Capay. In summary, all genotypes were relatively stable

253　in GPC, with no significant differences observed in slopes.

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

255　　Five traits—yield (kg ha^{-1}), GPC (%), individual-grain weight and grain size (plump and

256　thins)—were examined in a linear mixed effect modeling framework. Genotype, location and

257　year-wise averages are reported in Table S2. The samples from 2020-21 season had relatively

258　smaller grain size and higher onset starch GTs (Table S2).

259　　The percentage of variance explained by the main and interaction effects of G, L, and Y

260　are shown in Table 1. For yield and GPC, the L:Y interaction accounted for 58% and 72% of the

261　variance, respectively. The largest variance in percentage of plump and thin grains was explained

262　by Y and G. The linear model that was fit for individual-grain weight had a large extent of

263　residual variance, potentially indicating that this trait is more dependent on specific management

264　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				

268

269   These results (Table 1) are in line with a previous genotype by environment study

270   conducted in Ethiopia (Bantayehu, 2013), where location explained the largest variance in grain

271   quality traits. Interestingly, in studies where the malt quality was assessed as opposed to grain

272   quality (like in the present study), the contribution of variance coming from G was larger than L

273   and/or Y (Nielsen and Munck, 2003; Laidig et al., 2017). This highlights the need for a deeper

274   understanding of how grain quality traits correlate with malt quality traits (e.g., total starch

275   extract %, total β-glucan content). Furthermore, a more in-depth characterization of grain and

276   malt quality parameters in a larger number of genotypes could also be worthwhile to inform

277   selection in earlier stages of the breeding process. Enabling such characterizations at a greater

278   scale and/or with higher throughput could have value to the industry and research community.

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

280   The main and interaction effects of G, L and Y on starch GT are shown in Table 2. For

281   onset GT, the largest percentage of variance was explained by Y, followed by G. For peak and

282   offset GT, the largest percentage of variance was explained by G and the L:Y interaction term,

283   respectively. In addition to the onset, peak and offset GT, it is also important to consider the GT

284 temperature range using the difference between onset and peak, and peak and offset GT. A

285 broader GT range will result in a wider DSC curve, which has been attributed to the presence of

286 more type A starch granules that have been packed heterogeneously (Vasanthan and Bhatty,

287 1996; Suh et al., 2004). Similar to individual-grain weight, the residual variance of the difference

288 between onset and peak GT was large, suggesting that there could be variance associated with

289 specific management factors that were not explicitly tested in this model.

290 **Table 2. Main and interaction effects of genotype (G), location (L), and year (Y) on starch**

291 **GT using linear mixed effects models. Units are °C for all of the traits presented in this**

292 **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				

293

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

304 (Langenaeken et al., 2019). These smaller starch granules are often developed due to changes in

305 starch biosynthesis during grain development that are triggered by drought (Gous et al., 2015).

306 Hence, a large extent of variance in the difference between peak and offset GT being explained

307 by Y could prove to be problematic for the malting and brewing industries.

308 **Effect of nitrogen fertilization on malting barley productivity and quality**

309   In order to study the effect of added N on malting barley quality and starch gelatinization,

310 dataset from the 2016-17 season consisting of three locations, two genotypes and multiple N

311 treatments applied prior to planting or at tillering growth stage was leveraged. For the Davis, Rio

312 Vista and Tulelake sites in this experiment, the average grain yield/GPC were 4478 kg ha^{-1}/9.8%,

313 3160 kg ha^{-1}/12.6% and 7178 kg ha^{-1}/12.3%, respectively. The results of linear mixed-effects

314 modeling in this dataset indicated that L and G accounted for the largest variance in all starch GT

315 traits, except the difference between onset and peak GT (Table 3). Added N levels at tillering

316 accounted for 18% of the variance in this trait. However, pre-plant added N levels did not

317 account for any variance in GT traits. This could be because of the tillering stage being closer to

318 growth stages related to heading and grain-fill when the starch granules in the grain are

319 developed. L accounted for the largest variance in percentage of plump and thin grains.

320 **Table 3. Effect of added nitrogen levels pre-planting in kg ha^{-1} (N$_1$), at tillering in kg ha^{-1}**

321 **(N$_2$), location (L) and genotype (G) on malting barley productivity and quality using linear**

322 **mixed effects models. Units are °C for all of the GT-related traits presented in this table.**

323 **'Res' refers to residuals.**

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

	% 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

	% Explained	Variance	Std. dev.		% Explained	Variance	Std. dev.
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

324

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

338 applications in winter wheat (Zebarth and Sheard, 1992), with low and high precipitation levels

339 influencing the effects of N timing and rate on grain yields. In our study, soil water availability

340 to the crops at the different locations varied dramatically. At each site, N fertilizer was applied

341 prior to/in coincidence with precipitation and/or irrigation to ensure N incorporation into the soil

342 during the fertilization event itself. However, this does not imply that water supply was sufficient

343 to meet crop demand for the remainder of the season. It is likely that water-limitation later in the

344 season impacted seasonal N uptake patterns and prevented efficient/complete seasonal N uptake

345 at some of the locations. Therefore, one hypothesis is that the applied N levels did not contribute

346 to considerable variance in yield and GPC in the current study due to large differences in soil

347 water availability among locations and potential water limitations impacting N assimilation.

348 While our results indicate that G and L contribute to a greater degree of variance in comparison

349 to N fertilizer application, this dataset was only from one year (2016-17). A dataset with a higher

350 resolution (including samples from multiple years) might be required to study interaction effects

351 between location and N levels.

352 **Trait relationships (correlations and principal component analysis)**

353   Pearson correlations were examined between the malting barley productivity and quality

354 traits studied herein (Figure 2A). Correlations discussed here are indicated using colored circles

355 and were statistically significant based on a 95% asymptotic confidence interval using Fisher's Z

356 transform. Yield was positively correlated with plump % ($r = 0.37$) and the difference between

357 peak and offset GT (0.37), but was negatively correlated with thin % (-0.31) and peak GT (-

358 0.20). GPC was negatively correlated with plump % (-0.41) and yield (-0.24). Peak GT was

359 negatively correlated with yield (-0.24) and plump % (-0.15), but was positively correlated with

360 individual-grain weight (0.18).

A

363

C

364
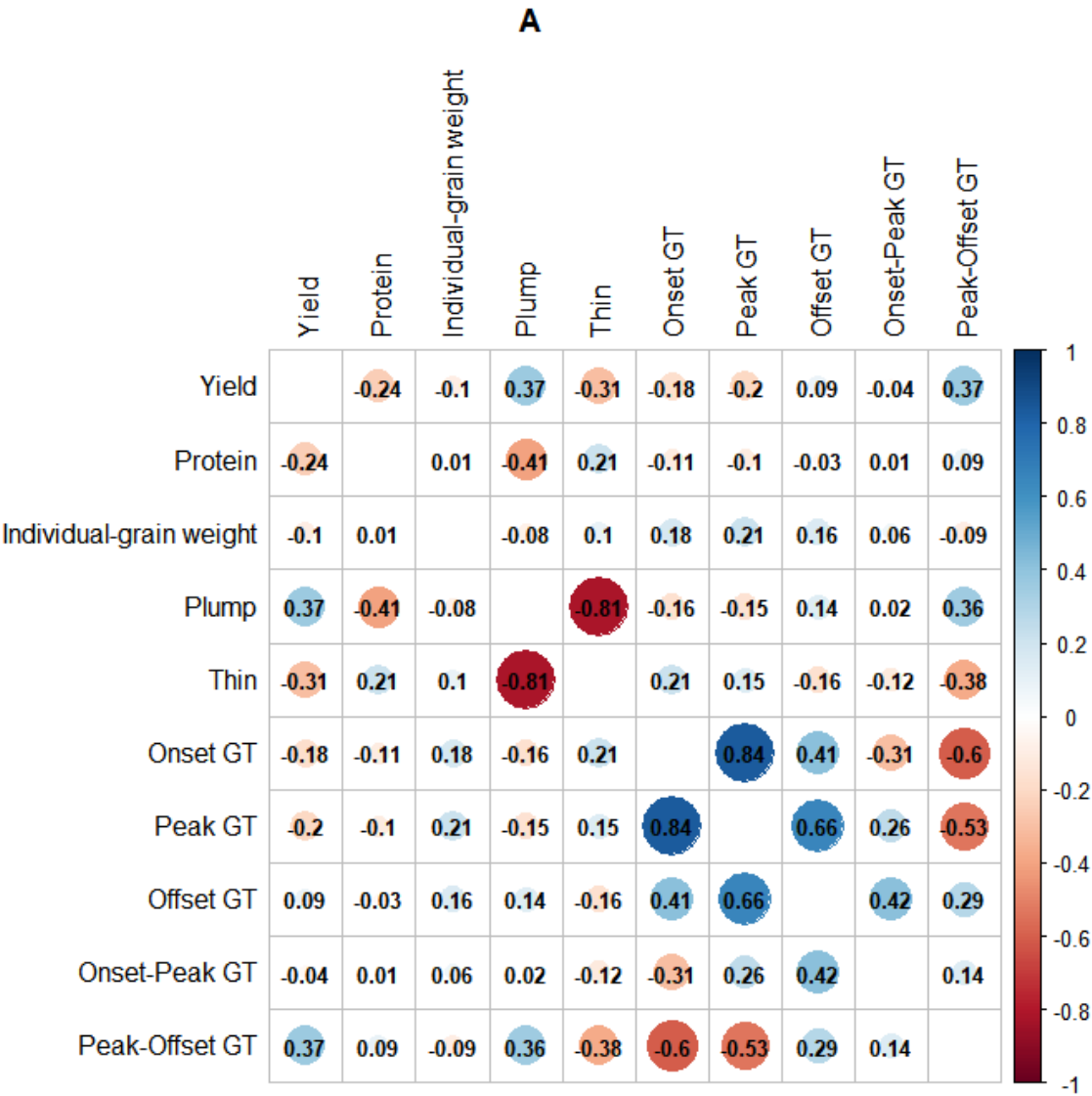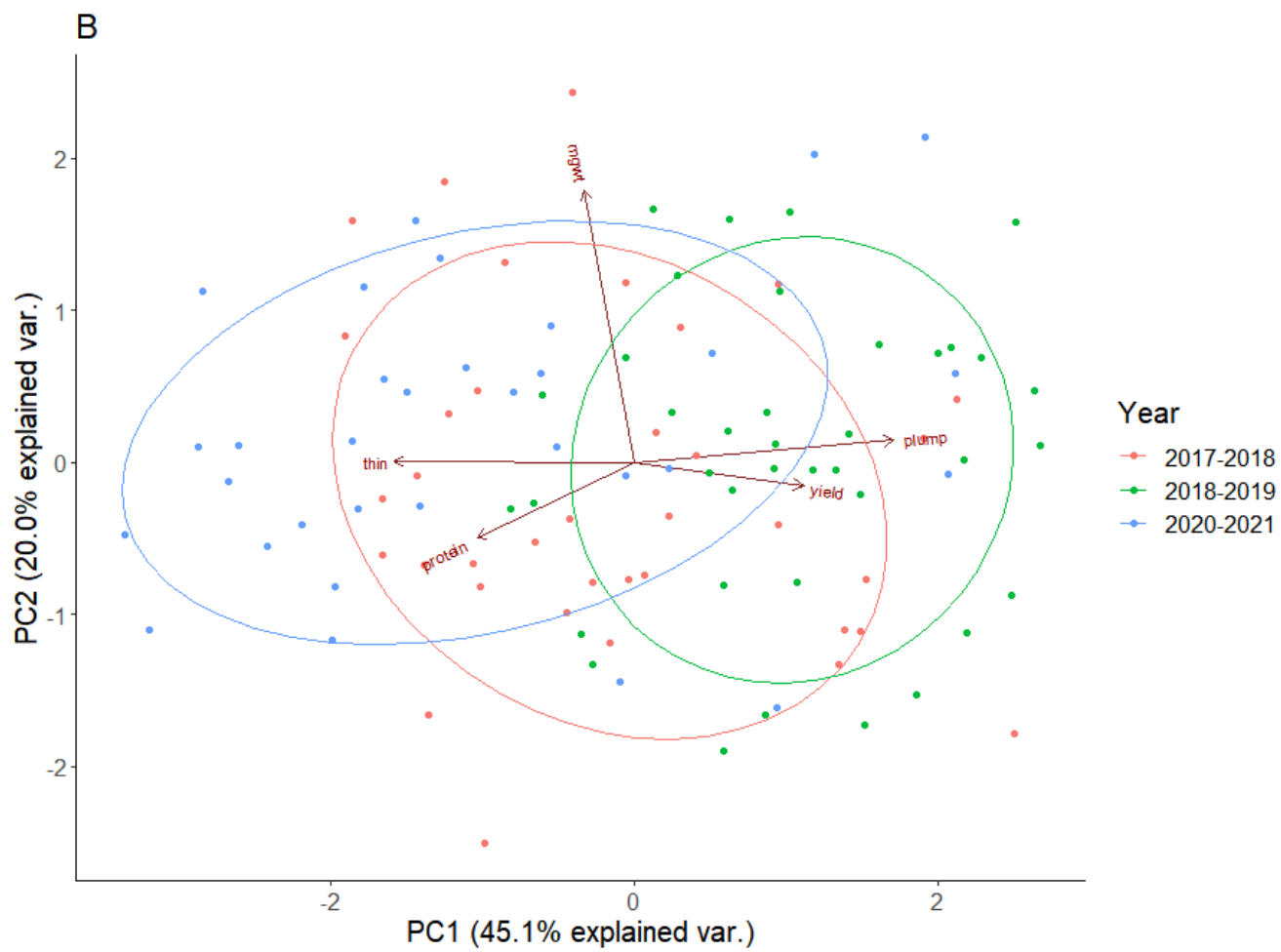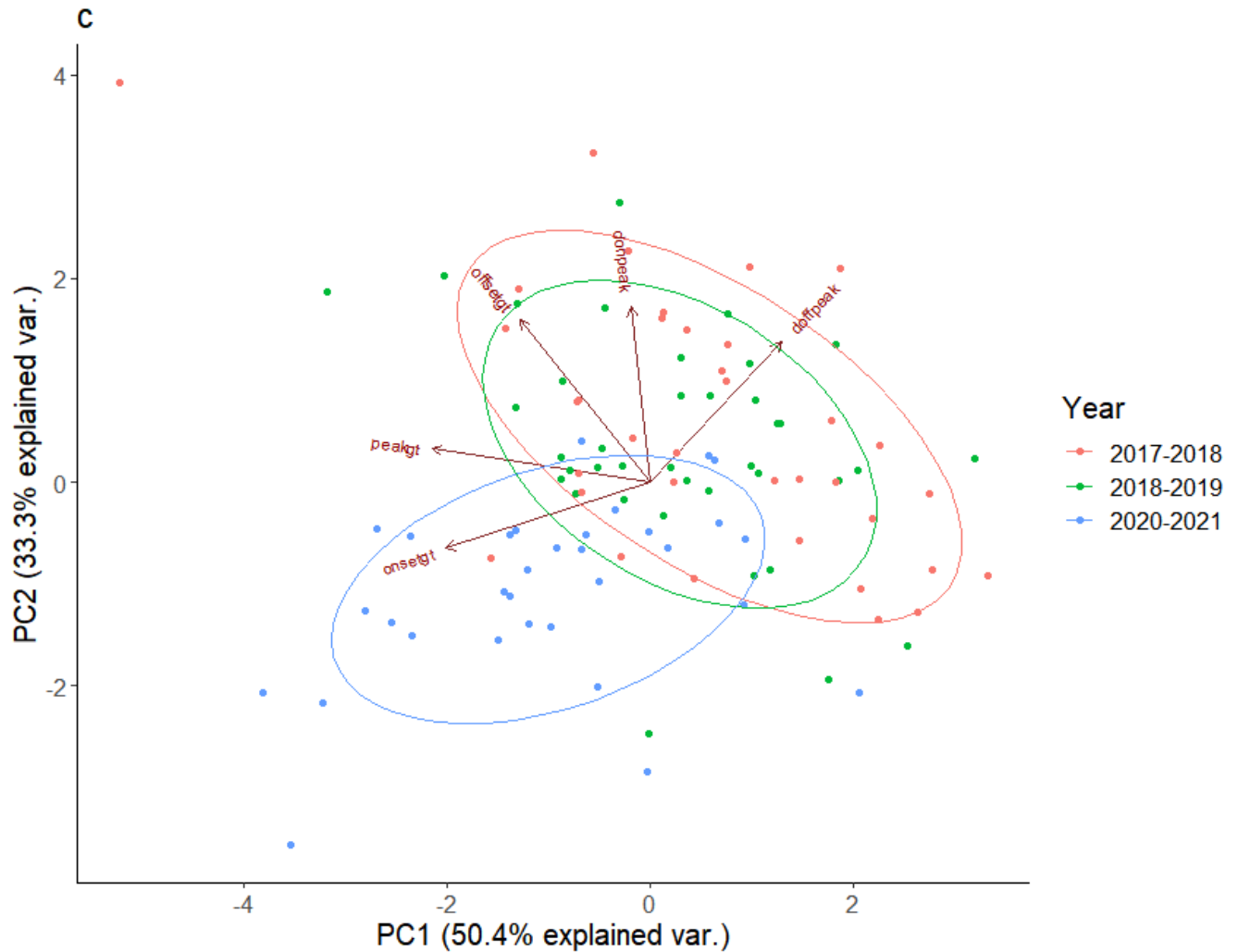
Figure 2. A) Pearson correlation coefficient (*r*) matrix between malting barley productivity and

quality traits. Colored circles indicate significant correlations based on a 95% asymptotic

confidence interval using Fisher's Z transform; Year based biplots for B) productivity and

quality traits PCs 1 and 2; C) GT traits PCs 1 and 2. Vectors represent yield in kg ha^{-1} (*yield*),

GPC (*protein*), percentage of plump (*plump*) and thin (*thin*) grains, individual-grain weight in

mg (*mgwt*), onset GT (*onsetgt*), peak GT (*peakgt*), offset GT (*offsetgt*), difference between onset

and peak GT (*donpeak*), and the difference between peak and offset GT (*doffpeak*). Normal

372   confidence ellipses based on multivariate t-distribution were drawn with 95% confidence

373   intervals for all biplots.

374

375   The correlation between yield and GPC is generally accepted to be negative in malting

376   barley (Fox, 2010). However newer studies have shown that potential explanations for this

377   negative correlation could be due to nitrogen availability (Magliano et al., 2014) and/or tiller

378   formation (Hu et al., 2021). The negative correlation between peak GT and percentage of plump

379   grains is expected as it has been previously established that plump grains also contain an overall

380   higher extractable starch content, therefore requiring a higher peak GT to hydrolyze the starch

381   (Andersson et al., 1999; Vahamidis et al., 2022).

382   The difference between peak and offset GT showed a strong negative correlation with the

383   onset GT (-0.60), peak GT (-0.53) and thin % (-0.38), but a positive correlation with yield (0.37)

384   and plump % (0.36). This trait (i.e., difference between peak and offset GT) has not been

385   previously reported, and suggests that some alternative endosperm parameter not assessed in this

386   study could be causing a delay in the gelatinization of starch above the peak temperature. One

387   potential explanation to this correlation could be attributed to the proportion of A-type and B-

388   type starch granules within the endosperm (Goering et al., 1973; Vasanthan and Bhatty, 1996).

389   The smaller B-type granules which are developed later in the grain filling process have been

390   previously shown to gelatinize more slowly than A-type granules (Karlsson et al., 1983;

391   Langenaeken et al., 2019). Hence it is possible that the samples with a higher difference between

392   the peak and offset GT assessed in this study could contain a higher proportion of B-type

393   granules than A-type granules. Another possibility could be the variation in hordein (major seed

394　storage proteins in the endosperm) levels which could impact the accessibility of starch granules

395　to starch-degrading enzymes (Wenwen et al., 2019). Further research on starch granule

396　proportions and hordein content will enable a more comprehensive understanding of this

397　complex relationship between starch GT and grain quality.

398　　　PCA biplots were used to visualize the relationships among traits across three years

399　(Figure 2B and 2C). Normal confidence ellipses based on multivariate t-distribution were drawn

400　with 95% confidence intervals for each year. For productivity and quality traits, the first, second,

401　and third principal components (PCs) explained 45%, 20% and 16.9% of the total variance,

402　respectively. For the starch GT traits, the first and second PCs explained 55.4% and 33.3% of the

403　total variance, respectively. The 2020-21 season samples formed a partially distinct cluster,

404　mainly discriminated by high percentage of thin grains (Figure 2B) and high onset GT (Figure

405　2C).

406　**Gelatinization profiles (differential scanning calorimetry curves)**

407　　　The average values of the malting barley productivity and grain quality traits based on

408　year, location and genotype were examined to assess key differences (Table S2). The 2020-21

409　year had relatively higher average values for onset, peak and offset GT and lower percentage of

410　plump grains. Figure 3 illustrates how the starch gelatinization curves for UC Tahoe and UC

411　Capay differ across the four seasons. On average, the onset GT was higher for the 2020-21

412　season in comparison to other seasons for both UC Tahoe and UC Capay. This was also in line

413　with the PCA results observed for this season (Figure 2C).

414

A

415

B
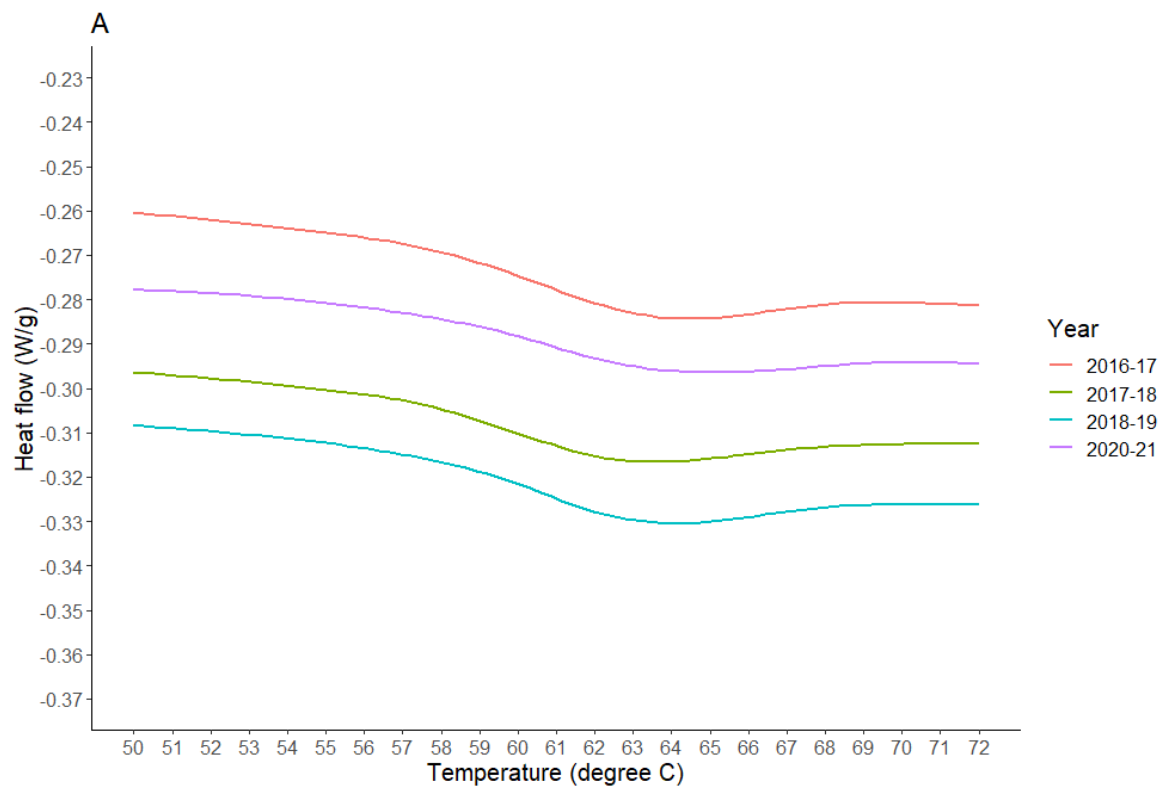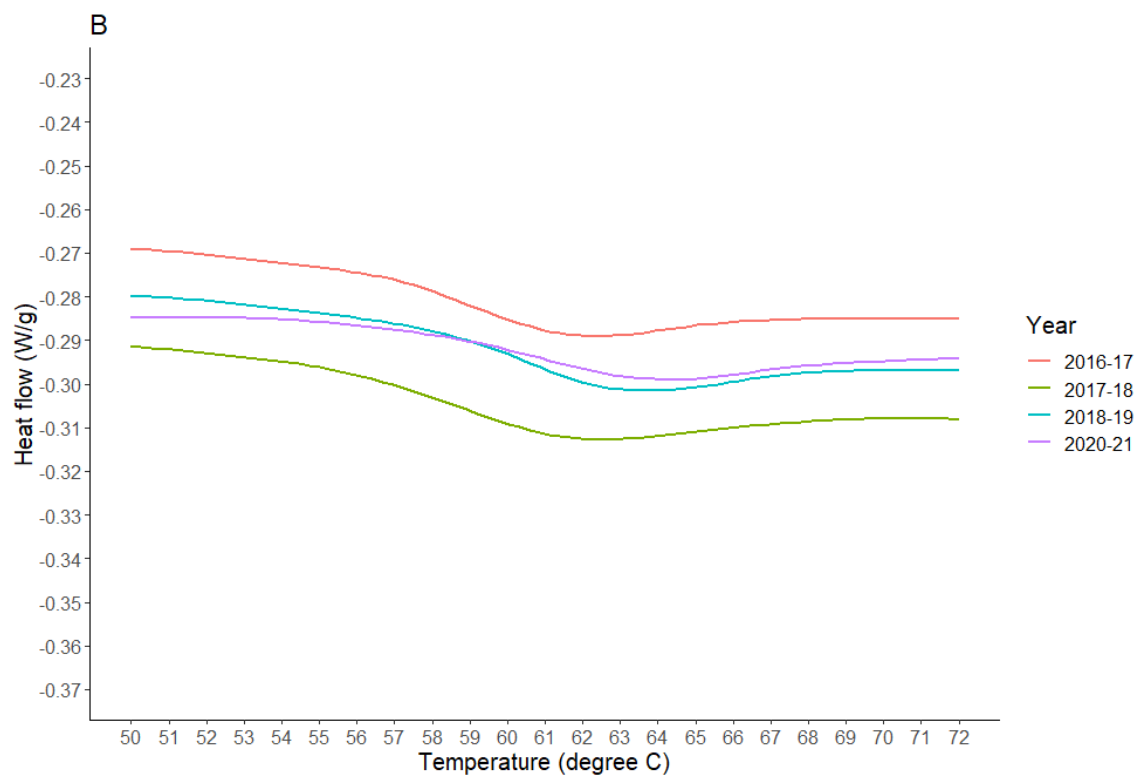
416

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

439 variance in onset and offset starch GT, respectively, but the largest variance in peak GT was

440 explained by G. Added N levels accounted for only 5% variance in GPC, but accounted for 18%

441 of variance in the difference between onset and peak GT. The effect of added N levels was

442 minimal for all other traits. Finally, PCA of the same dataset used for the FW regression shows

443 that the 2020-21 season formed partially distinct clusters, segregated by a high percentage of thin

444 grains and high onset GT. These findings illustrate the critical role of G, L and Y in determining

445 malting barley productivity and grain quality in California.

446

447

448 **SUPPLEMENTAL MATERIAL**

449 The supplemental material includes tables detailing coordinates, climatic and

450 management information of the locations (Table S1), average trait values by year, location and

451 genotype (Table S2) and pre and post heading water levels for locations per year (Table S3). It

452 also includes a map of California where the field trials were conducted (Figure S1). Other figures

453 (Figure S2 and S3) are scree plots corresponding to the biplots in the main manuscript, and

454 biplots corresponding to PCs 1 and 3; and 2 and 3. PCA biplots based on location are shared in

455 Figure S4. It also includes the R script and associated data files that were used for data analysis

456 and visualization.

457

458

459

REFERENCES

460 AMBA. 2022. Barley Production Report August 12, 2022. AMBA.

462 American Society of Brewing Chemists. 1984. ASBC Methods of Analysis Barley 7.
463   https://www.asbcnet.org/Methods/BarleyMethods/Pages/default.aspx (accessed 1 November
464   2022).

465 American Society of Brewing Chemists. 2012. ASBC Methods of Analysis Malt-2.
466   http://dx.doi.org/10.1094/ASBCMOA-Malt-2 (accessed 23 March 2022).

467 Andersson, A.A.M., C. Elfverson, R. Andersson, S. Regnér, and P. Åman. 1999. Chemical and
468   physical characteristics of different barley samples. Journal of the Science of Food and
469   Agriculture.

470 Bantayehu, M. 2013. Study on malting barley genotypes under diverse Agroecologies of north
471   western Ethiopia. Afr. J. Plant Sci. 7(11): 548–557. doi: 10.5897/AJPS10.068.

472 Bates, D., M. Maechler, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4.
473   Journal of Statistical Software 67(1): 1–48.

474 del Blanco, I.A., J.M. Hegarty, and J. Dubcovsky. 2022. Registration of 'UC-Capay', a low-
475   grain-protein, non–glycoside nitrile producing, California-adapted, two-rowed spring malting
476   barley. J. Plant Regist. doi: 10.1002/plr2.20240.

477 Briggs, D.E. 1998. Malts and Malting. Springer US.

478 California department of water resources. 2021. Water year 2021: An extreme year. California
479   department of water resources.

480 Canadian Food Inspection Agency. 2007. Barley: List of Varieties Which are Registered in
481   Canada. https://inspection.canada.ca/active/netapp/regvar/regvare.aspx?id=2484#shr-pg0
482   (accessed 3 July 2022).

483 Choi, H., A. Esser, and K.M. Murphy. 2020. Genotype × environment interaction and stability of
484   β-glucan content in barley in the Palouse region of eastern Washington. Crop Sci. 60(5):
485   2500–2510. doi: 10.1002/csc2.20181.

486 Evans, E., B. van Wegen, Y. Ma, and J. Eglinton. 2003. The Impact of the Thermostability of α-
487   Amylase, β-Amylase, and Limit Dextrinase on Potential Wort Fermentability. ASBCJ 61(4):
488   210–218. doi: 10.1094/ASBCJ-61-0210.

489 Finlay, K.W., and G.N. Wilkinson. 1963. The Analysis of Adaptation in a Plant-Breeding
490   Programme. Aust J Agric Res 14: 742–54.

Fox, G., K. Onley-Watson, Ros. Ferguson, A. Skerman, D. Poulsen, et al. 2001. Grain and Malt Quality Evaluation from a Structured Environmental Trial. The Regional Institute of Online Publishing

Fox, G.P., B. Osborne, J. Bowman, A. Kelly, M. Cakir, et al. 2007. Measurement of genetic and environmental variation in barley (Hordeum vulgare) grain hardness. J. Cereal Sci. 46(1): 82–92. doi: 10.1016/j.jcs.2006.12.003.

Fox, G.P., M. Staunton, E. Agnew, and B. D'Arcy. 2019. Effect of varying starch properties and mashing conditions on wort sugar profiles. J. Inst. Brew. 125(4): 412–421. doi: 10.1002/jib.585.

Fox, J., and S. Weisberg. 2019. An R Companion to Applied Regression. 3rd ed. Sage, Thousand Oaks, CA.

Fox, G.P. 2010. Chemical Composition in Barley Grains and Malt Quality. In: Zhang, G. and Li, C., editors, Genetics and Improvement of Barley Malt Quality. Zhejiang University Press, Springer: Hangzhou, China

Fredriksson, H., J. Silverio, R. Andersson, A.C. Eliasson, and P. Åman. 1998. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. Carbohydr. Polym. 35(3–4): 119–134. doi: 10.1016/S0144-8617(97)00247-6.

Gallagher, L.W., R. Silberstein, L. Prato, and H. Vogt. 2020. 'Butta 12', a two-rowed malting barley adapted to the California Central Valley with proven floor-malting success and craft brewer acceptance. J. Plant Regist. 14(3): 250–265. doi: 10.1002/plr2.20067.

George, N., and M. Lundy. 2019. Quantifying Genotype × Environment Effects in Long-Term Common Wheat Yield Trials from an Agroecologically Diverse Production Region. Crop Sci. 59(5): 1960–1972. doi: 10.2135/cropsci2019.01.0010.

Giordano, N., V.O. Sadras, and R.P. Lollato. 2023. Late-season nitrogen application increases grain protein concentration and is neutral for yield in wheat. A global meta-analysis. Field Crops Res. 290: 108740. doi: 10.1016/j.fcr.2022.108740.

Goering, K.J., D.H. Fritts, and R.F. Eslick. 1973. A study of starch granule size and distribution in 29 barley varieties. Starch/Stärke 25(9): 297–302. doi: 10.1002/star.19730250904.

Gous, P.W., F. Warren, O.W. Mo, R.G. Gilbert, and G.P. Fox. 2015. The effects of variable nitrogen application on barley starch structure under drought stress. J. Inst. Brew. 121(4): 502–509. doi: 10.1002/jib.260.

Greenwood, C.T., and J. Thomson. 1959. A comparison of the starches from barley and malted barley. J. Inst. Brew. 65(4): 346–353. doi: 10.1002/j.2050-0416.1959.tb01470.x.

525 Gujral, H.S., P. Sharma, H. Kaur, and J. Singh. 2013. Physiochemical, Pasting, and Thermal
526   Properties of Starch Isolated from Different Barley Cultivars. International Journal of Food
527   Properties 16(7): 1494–1506. doi: 10.1080/10942912.2011.595863.

528 Halstead, M., C. Morrissy, S. Fisk, G. Fox, P. Hayes, et al. 2022. Barley grain protein is
529   influenced by genotype, environment, and nitrogen management and is the major driver of
530   malting quality. Crop Sci. doi: 10.1002/csc2.20842.

531 Hochman, A., F. Marra, G. Messori, J.G. Pinto, S. Raveh-Rubin, et al. 2021. ESD reviews:
532   extreme weather and societal impacts in the eastern mediterranean. doi: 10.5194/esd-2021-55.

533 Hu, Y., G. Barmeier, and U. Schmidhalter. 2021. Genetic variation in grain yield and quality
534   traits of spring malting barley. Agronomy 11(6): 1177. doi: 10.3390/agronomy11061177.

535 Izydorczyk, M.S., A.W. MacGregor, and C.G. Billiaderis. 2001. Effects of Malting on Phase
536   Transition Behaviour of Starch in Barley Cultivars with Varying Amylose Content. J. Inst.
537   Brew. 107(2): 119–128. doi: 10.1002/j.2050-0416.2001.tb00084.x.

538 Jackson, L., B. Fernandez, H. Meister, and M. Spiller. 2006. Small grain production manual.
539   University of California, Agriculture and Natural Resources.

540 Jaiswal, S., M. Båga, G. Ahuja, B.G. Rossnagel, and R.N. Chibbar. 2014. Development of barley
541   (Hordeum vulgare L.) lines with altered starch granule size distribution. J. Agric. Food Chem.
542   62(10): 2289–2296. doi: 10.1021/jf405424x.

543 Källman, A., V. Vamadevan, E. Bertoft, K. Koch, K. Seetharaman, et al. 2015. Thermal
544   properties of barley starch and its relation to starch characteristics. Int. J. Biol. Macromol. 81:
545   692–700. doi: 10.1016/j.ijbiomac.2015.08.068.

546 Kandic, V., D. Dodig, M. Secanski, S. Prodanovic, G. Brankovic, et al. 2019. Grain yield,
547   agronomic traits, and protein content of two- and six-row barley genotypes under terminal
548   drought conditions. Chilean J. Agric. Res. 79(4): 648–657. doi: 10.4067/S0718-
549   58392019000400648.

550 Kanter, J., N. Clark, M.E. Lundy, V. Koundinya, M. Leinfelder-Miles, et al. 2021. Top
551   management challenges and concerns for agronomic crop production in California: Identifying
552   critical issues for extension through needs assessment. Agron. J. 113(6): 5254–5270. doi:
553   10.1002/agj2.20897.

554 Karlsson, R., R. Olered, and A.C. Eliasson. 1983. Changes in starch granule size distribution and
555   starch gelatinization properties during development and maturation of wheat, barley and rye.
556   Starch/Stärke 35(10): 335–340. doi: 10.1002/star.19830351002.

557 Kassambara, A., and F. Mundt. 2020. Factoextra: Extract and Visualize the Results of
558   Multivariate Data Analyses. R Package Version 1.0.7. https://CRAN.R-
559   project.org/package=factoextra (accessed 25 June 2022).

560 Laidig, F., H.-P. Piepho, D. Rentel, T. Drobek, and U. Meyer. 2017. Breeding progress,
561 genotypic and environmental variation and correlation of quality traits in malting barley in
562 German official variety trials between 1983 and 2015. Theor. Appl. Genet. 130(11): 2411–
563 2429. doi: 10.1007/s00122-017-2967-4.

564 Langenaeken, N.A., C.F. De Schepper, D.P. De Schutter, and C.M. Courtin. 2019. Different
565 gelatinization characteristics of small and large barley starch granules impact their enzymatic
566 hydrolysis and sugar production during mashing. Food Chem. 295: 138–146. doi:
567 10.1016/j.foodchem.2019.05.045.

568 Legge, W.G., J.R. Tucker, T.G. Fetch, S. Haber, J.G. Menzies, et al. 2014. AAC Synergy barley.
569 Can. J. Plant Sci. 94(4): 797–803. doi: 10.4141/cjps2013-307.

570 Lian, L., and G. de Los Campos. 2015. FW: An R Package for Finlay-Wilkinson Regression that
571 Incorporates Genomic/Pedigree Information and Covariance Structures Between
572 Environments. G3 (Bethesda) 6(3): 589–597. doi: 10.1534/g3.115.026328.

573 Limagrain Cereal Seeds. 2022. LCS Odyssey malting barley.
574 https://limagraincerealseeds.com/malting-barley-seed/lcs-odyssey/ (accessed 1 November
575 2022).

576 Lindblad, B. 2022. tidyUSDA: A Minimal Tool Set for Gathering USDA Quick Stat Data for
577 Analysis and Visualization. R package.

578 Magliano, P.N., P. Prystupa, and F.H. Gutiérrez-Boem. 2014. Protein content of grains of
579 different size fractions in malting barley. J. Inst. Brew. doi: 10.1002/jib.161.

580 de Mendiburu, F., and M. Yaseen. 2020. agricolae: Statistical Procedures for Agricultural
581 Research. R package version 1.4.0. https://myaseen208.github.io/agricolae/https://cran.r-
582 project.org/package=agricolae (accessed 24 June 2022).

583 Mohammadi, M., T.K. Blake, A.D. Budde, S. Chao, P.M. Hayes, et al. 2015. A genome-wide
584 association study of malting quality across eight U.S. barley breeding programs. Theor. Appl.
585 Genet. 128(4): 705–721. doi: 10.1007/s00122-015-2465-5.

586 Myllärinen, P., A.H. Schulman, H. Salovaara, and K. Poutanen. 1998. The effect of growth
587 temperature on gelatinization properties of barley starch. Acta Agriculturae Scandinavica,
588 Section B — Soil & Plant Science 48(2): 85–90. doi: 10.1080/09064719809362484.

589 Nelsen, T., M. Levinson, and M. Lundy. 2021a. 2020-21 Regional Common Wheat & Triticale,
590 Durum Wheat,and Barley Performance Tests in California. UC-ANR.

591 Nelsen, T.S., and M.E. Lundy. 2020. Canopy reflectance informs in-season malting barley
592 nitrogen management: An ex-ante classification approach. Agron. J. 112(6): 4705–4722. doi:
593 10.1002/agj2.20397.

594 Nelsen, T., J. Merz, G. Rosa, and M. Lundy. 2021b. The California Weather Web-Tool. The
595   California Weather Web-Tool. http://smallgrain-n-
596   management.plantsciences.ucdavis.edu/weather/ (accessed 14 October 2022).

597 Newman, C.W., and R.K. Newman. 1992. Nutritional aspects of barley seed structure and
598   composition.

599 Nielsen, J.P., and L. Munck. 2003. Evaluation of malting barley quality using exploratory data
600   analysis. I. Extraction of information from micro-malting data of spring and winter barley. J.
601   Cereal Sci. 38(2): 173–180. doi: 10.1016/S0733-5210(03)00023-7.

602 Patindol, J., G. Mendez-Montealvo, and Y.-J. Wang. 2012. Starch properties of malted barley in
603   relation to real degree of fermentation. Starch/Stärke 64(7): 517–523. doi:
604   10.1002/star.201100171.

605 Peterson, C., C. Pittelkow, M. Lundy, and J. Collins. 2022. Exploring the Potential for Water-
606   Limited Agriculture in the San Joaquin Valley. Public Policy Institute of California.

607 Prystupa, P., G. Ferraris, L. Ventimiglia, T. Loewy, L. Couretot, et al. 2018. Environmental
608   control of malting barley response to nitrogen in the Pampas, Argentina. Int. J. Plant Prod.
609   12(2): 127–137. doi: 10.1007/s42106-018-0013-3.

610 Przulj, N., V. Momcilovic, J. Simic, and M. Mirosavljevic. 2014. Effect of growing season and
611   variety on quality of spring two-rowed barley. Genetika 46(1): 59–73. doi:
612   10.2298/GENSR1401059P.

613 Pycia, K., D. Gałkowska, L. Juszczak, T. Fortuna, and T. Witczak. 2015. Physicochemical,
614   thermal and rheological properties of starches isolated from malting barley varieties. J. Food
615   Sci. Technol. 52(8): 4797–4807. doi: 10.1007/s13197-014-1531-3.

616 RStudio Team. 2022. RStudio: Integrated Development Environment for R. PBC, Boston, MA.

617 R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for
618   Statistical Computing, Vienna, Austria.

619 Searle, S.R., F.M. Speed, and G.A. Milliken. 1980. Population marginal means in the linear
620   model: an alternative to least squares means. The American Statistician 34(4): 216–221. doi:
621   10.1080/00031305.1980.10483031.

622 Suh, D.S., T. Verhoeven, K. Denyer, and J. Jane. 2004. Characterization of Nubet and Franubet
623   barley starches. Carbohydr. Polym. 56(1): 85–93. doi: 10.1016/j.carbpol.2003.12.005.

624 Tester, R.F., and W.R. Morrison. 1990. Swelling and gelatinization of cereal starches. I. Effects
625   of amylopectin, amylose, and lipids. Cereal chem 67(6): 551–557.

626 UC-ANR. 2020. Barley statewide variety results. https://ucanr.edu/sites/small-
627   grains/files/336501.pdf (accessed 13 May 2022).

628 Vahamidis, P., A. Stefopoulou, V. Kotoulas, P. Bresta, D. Nikolopoulos, et al. 2022. Grain size
629   variation in two-rowed malt barley under Mediterranean conditions: Phenotypic plasticity and
630   relevant trade-offs. Field Crops Res. 279: 108454. doi: 10.1016/j.fcr.2022.108454.

631 Vasanthan, T., and R.S. Bhatty. 1996. Physicochemical properties of small- and large-granule
632   starches of waxy, regular, and high-amylose barleys. Cereal chemistry (USA).

633 Wei, T., and V. Simko. 2021. R package "corrplot": Visualization of a Correlation Matrix
634   (Version 0.92). https://github.com/taiyun/corrplot (accessed 25 June 2022).

635 Wenwen, Y., K. Tao, M.J. Gidley, G.P. Fox, and R.G. Gilbert. 2019. Molecular brewing:
636   Molecular structural effects involved in barley malting and mashing. Carbohydr. Polym. 206:
637   583–592. doi: 10.1016/j.carbpol.2018.11.018.

638 Wesenberg, D.M., R.M. Hayes, H.C. McKay, N.N. Standridge, E.D. Goplin, et al. 1974.
639   Registration of klages barley (reg. no. 138). Crop Sci. 14(2): 337–338. doi:
640   10.2135/cropsci1974.0011183X001400020054x.

641 Wickham, H. 2007. Reshaping Data with the reshape Package. Journal of Statistical Software
642   21(12): 1–20.

643 Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

644 Wickham et al. 2019. Welcome to the tidyverse. Journal of Open Source Software 4(43): 1686.

645 Wuest, S.B., and K.G. Cassman. 1992. Fertilizer-Nitrogen Use Efficiency of Irrigated Wheat: I.
646   Uptake Efficiency of Preplant versus Late-Season Application. Agron. J. 84(4): 682–688. doi:
647   10.2134/agronj1992.00021962008400040028x.

648 You, S., and M.S. Izydorczyk. 2007. Comparison of the physicochemical properties of barley
649   starches after partial α-amylolysis and acid/alcohol hydrolysis. Carbohydr. Polym. 69(3): 489–
650   502. doi: 10.1016/j.carbpol.2007.01.002.

651 Zebarth, B.J., and R.W. Sheard. 1992. Influence of rate and timing of nitrogen fertilization on
652   yield and quality of hard red winter wheat in Ontario. Can. J. Plant Sci. 72(1): 13–19. doi:
653   10.4141/cjps92-002.

654 Zhou, B., Z. Jin, P. Schwarz, and Y. Li. 2020. Impact of genotype, environment, and malting
655   conditions on the antioxidant activity and phenolic content in US malting barley. Fermentation
656   6(2): 48. doi: 10.3390/fermentation6020048.

Table S1. Summary of locations and genotypes tested within California. All temperatures and water levels correspond to the entire fall growing season (Nov-June). Irrigation and precipitation levels are in mm.

Year	Location	Latitude	Longitude	Planting Date mm/dd/yyyy	Minimum Temp (°C)	Maximum Temp (°C)	Avg Temp (°C)	Total Irrigation	Total Precipitation	Total water	Genotype	Fertilizer applied (kg/ha)
2016-17	Davis	38.53	-121.77	11/15/2016 (Nelsen & Lundy, 2020)	7.88	19.98	13.93	0	835.7	835.7	UC Tahoe	57.4, 75.3, 82.1, 82.2
2017-18	Davis	38.54	-121.78	11/21/2017	7.19	21.26	14.23	190.5	315	505.5	LCS Odyssey	112
											AAC Synergy	
											UC Tahoe	
											UC Capay	
											OSU Full Pint	
											LCS Genie	
											UC Butta 12	
											CDC Copeland	
											UC B9K94	
											Klages	
2018-19	Davis	38.53	-121.77	12/13/2018	8.09	20.71	14.4	63.5	741.7	805.2	LCS Odyssey	101
											UC Tahoe	
											LCS Genie	
											UC Butta 12	
											UC B9K94	
											UC 1911	
											OSU Full Pint	
											CDC Copeland	

Year	Location	Lat	Long	Date							Variety	
											UC Capay	
											AAC Synergy	
											Klages	
2020-21	Davis	38.53	-121.77	12/11/2020 (Nelsen & Lundy, 2020)	6.79	21.37	14.08	0	160	160	UC Tahoe	67
											LCS Genie	
											UC Capay	
											AAC Synergy	
											CDC Copeland	
											LCS Odyssey	
											UC 1412	
											UC 1911	
2016-17	Rio Vista	38.14	-121.74	11/16/2016 (Nelsen & Lundy, 2020)	5.98	19.26	12.62	0	594.4	594.4	UC Tahoe	27.4, 57.4, 78.8, 109.5, 109.6
2016-17	Tulelake	41.96	-121.47	05/05/2017 (Nelsen & Lundy, 2020)	6.68	26.59	16.64	290	287.1	577.1	UC Tahoe	27.4, 54.8, 57.4, 75.3, 82.1, 150.6
											UC Capay	0, 60, 90, 120
2020-21	Yolo Region site 2	38.8	-122.05	12/10/2020 (Nelsen & Lundy, 2020)	7.7	23.78	15.74	0	152.4	152.4	UC Tahoe	56
											LCS Genie	
											UC Capay	
											AAC Synergy	
											CDC Copeland	
											LCS Odyssey	
											UC 1412	
											UC 1911	
2017-18	Fresno	36.34	-120.11	11/29/2017	6.35	22.65	14.5	151.6	106.7	258.3	UC Capay	56
											OSU Full Pint	

Year	Location	Lat	Lon	Date							Varieties	
											LCS Odyssey UC Tahoe LCS Genie UC Butta 12 AAC Synergy CDC Copeland Klages	
2018-19	Fresno	36.34	-120.12	12/12/2018	7.42	21.92	14.67	370.8	254	624.8	LCS Odyssey UC Tahoe UC Butta 12 AAC Synergy LCS Genie CDC Copeland OSU Full Pint UC Capay Klages	56
2020-21	Fresno	36.34	-120.11	11/18/2020 (Nelsen & Lundy, 2020)	7.97	25.01	16.49	139.7	116.8	256.5	UC Tahoe LCS Genie UC Capay AAC Synergy CDC Copeland LCS Odyssey UC 1412 UC 1911	112
2020-21	Imperial Valley	32.92	-115.33	12/08/2020 (Nelsen & Lundy, 2020)	11.82	30.68	21.25	442	27.9	469.9	UC Tahoe LCS Genie	56

2017-18											UC Capay	
											AAC Synergy	
											CDC Copeland	
											LCS Odyssey	
											UC 1412	
											UC 1911	
2017-18	Tehama	39.88	-122.36	12/15/2017	6.87	21.5	14.19	0	299.7	299.7	UC B9K94	125
											UC Butta 12	
											AAC Synergy	
											UC Capay	
											LCS Odyssey	
											LCS Genie	
											CDC Copeland	
											OSU Full Pint	
											UC Tahoe	
2018-19	Yolo Region site 3	38.8	-122.05	11/27/2018	8.98	20.45	14.72	0	739.2	739.2	UC Tahoe	107
											LCS Odyssey	
											UC Butta 12	
											OSU Full Pint	
											CDC Copeland	
											Klages	
											AAC Synergy	
											LCS Genie	
											UC Capay	
2017-18	Solano	38.15	-121.82	11/21/2017	3.88	18.85	11.37	0	322.6	322.6	LCS Odyssey	56

											AAC Synergy	
											UC B9K94	
											LCS Genie	
											UC Capay	
											CDC Copeland	
											UC Butta 12	
											UC Tahoe	
2018-19	Merced	37.14	-120.76	11/19/2018	7.51	21.37	14.44	177.8	299.7	477.5	LCS Odyssey	112
											LCS Genie	
											UC Tahoe	
											UC Butta 12	
											OSU Full Pint	
											AAC Synergy	
											CDC Copeland	
											Klages	
											UC Capay	

Table S2. Average productivity and quality traits by year, location and genotype. All GTs are in °C. * represents a significant difference from reference slope (p<0.05).

	Yield (kg ha⁻¹)	Yield stability (FW slope)	GPC (%)	GPC stability (FW slope)	Individual-grain weight (mg)	Plump (>2.78 mm) %	Onset GT	Peak GT	Offset GT	Difference between onset and peak GT	Difference between peak and offset GT
Year											
2016-17	5420		12.1		49	62	58.2	62.9	67.4	4.7	4.5
2017-18	4501		12.2		43	40	57.7	63.3	69.0	5.7	5.7
2018-19	5213		10.1		42	59	58.0	63.5	69.0	5.5	5.5
2020-21	3254		11.3		45	25	59.3	64.4	68.5	5.1	4.0
Location											
Davis	4942		11.5		44	47	57.8	63.2	68.2	5.4	5.0
Fresno	5419		11.3		45	53	58.5	63.6	69.2	5.1	5.6
Imperial Valley	3581		7.9		41	31	59.7	64.8	68.4	5.1	3.6
Merced	5387		11.4		36	54	57.3	62.7	68.2	5.4	5.6
Rio Vista	3160		12.6		41	51	57.3	62.2	67.1	4.9	4.9

Solano	2345		11.3		38	52	57.6	63.1	69.0	5.4	5.9
Tehama	3223		12.6		47	32	58.4	64.5	69.4	6.1	4.8
Tulelake Basin	7178		12.3		55	65	59.0	63.8	68.0	4.7	4.2
Yolo Region 2	2700		13.2		47	4	59.3	64.4	68.9	5.1	4.5
Yolo Region 3	3522		8.4		43	44	58.3	64.0	69.1	5.8	5.1
Genotype											
AAC Synergy	4065	0.94	10.8	0.90	41	48	57.6	63.3	68.6	5.7	5.4
CDC Copeland	3850	0.90	11.2	0.92	42	37	58.4	63.7	68.9	5.3	5.2
Klages	4294	0.54	11.6	1.10	46	38	57.7	63.1	68.8	5.4	5.7
LCS Genie	4676	1.03	11.8	1.03	45	35	59.1	64.4	69.2	5.3	4.8
LCS Odyssey	4968	1.28*	10.8	1.27	41	39	59.9	65.5	70.0	5.6	4.5
OSU Full Pint	5416	1.12	11.7	0.90	42	44	56.9	62.1	67.8	5.2	5.7
UC 1412	3285	1.20	11.2	1.33	49	9	59.6	64.6	68.4	5.1	3.8
UC 1911	3839	0.91	10.7	0.93	42	44	59.3	64.6	68.8	5.3	4.2

UC B9K94	4687	0.71	11.8	0.85	43	37	57.1	63.2	69.0	6.0	5.8
UC Butta 12	4714	0.94	11.6	0.78	44	78	57.8	63.8	69.8	6.0	6.0
UC Capay	4887	1.08	11.6	1.00	50	61	58.2	63.2	67.6	4.9	4.5
UC Tahoe	4864	1.09	11.5	0.82	44	49	57.9	62.9	67.9	5.0	5.0

Table S3. Irrigation and precipitation levels pre and post heading for all sites in seasons 17-18, 18-19 and 20-21. All water values are in mm.

Year	Location	Irrigation pre-heading (11/1 to 3/14)	Irrigation post-heading (3/15 to 6/30)	Total Irrigation	Precipitation pre-heading (11/1 to 3/14)	Precipitation post-heading (3/15 to 6/30)	Total Precipitation	Water pre-heading (11/1 to 3/14)	Water post-heading (3/15 to 6/30)	Total water
2017-18	Davis	190.5	0	190.5	208.3	106.7	315	398.8	106.7	505.5
2017-18	Fresno	151.6	0	151.6	55.9	50.8	106.7	207.5	50.8	258.3
2017-18	Solano	0	0	0	208.3	114.3	322.6	208.3	114.3	322.6
2017-18	Tehama	0	0	0	165.1	134.6	299.7	165.1	134.6	299.7
2018-19	Davis	63.5	0	63.5	589.3	152.4	741.7	652.8	152.4	805.2
2018-19	Fresno	264.2	106.6	370.8	200.7	53.3	254	464.9	159.9	624.8
2018-19	Yolo Region site 3	0	0	0	619.8	119.4	739.2	619.8	119.4	739.2
2018-19	Merced	177.8	0	177.8	246.4	53.3	299.7	424.2	53.3	477.5
2020-21	Davis	0	0	0	147.3	12.7	160	147.3	12.7	160
2020-21	Fresno	88.9	50.8	139.7	101.6	15.2	116.8	190.5	66	256.5

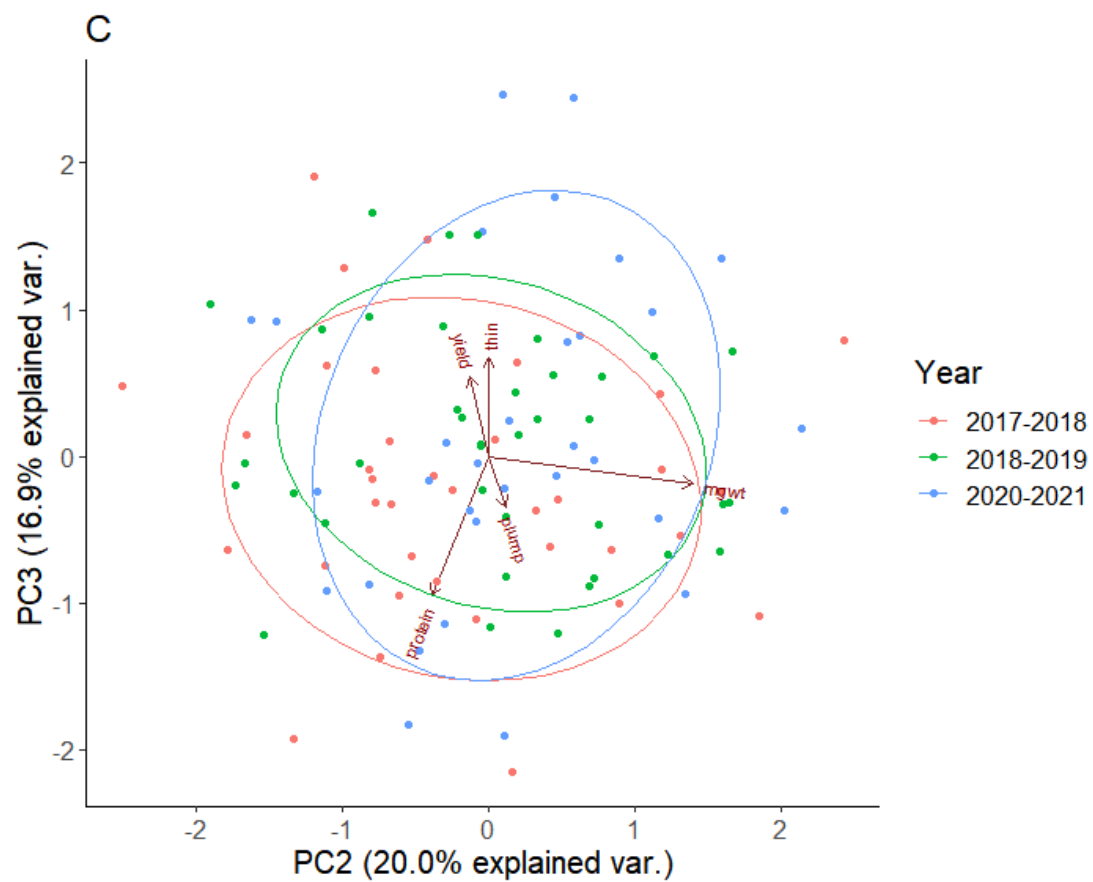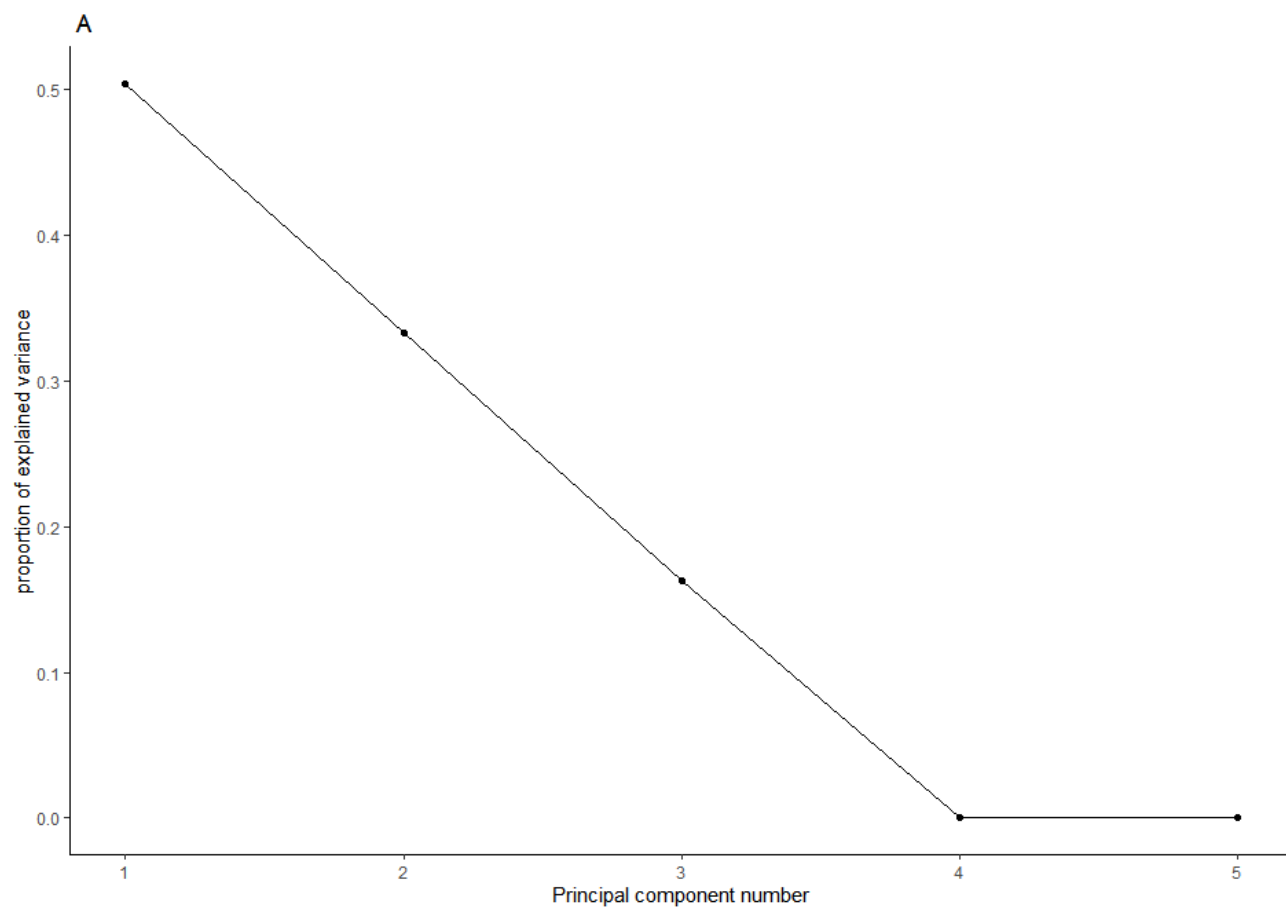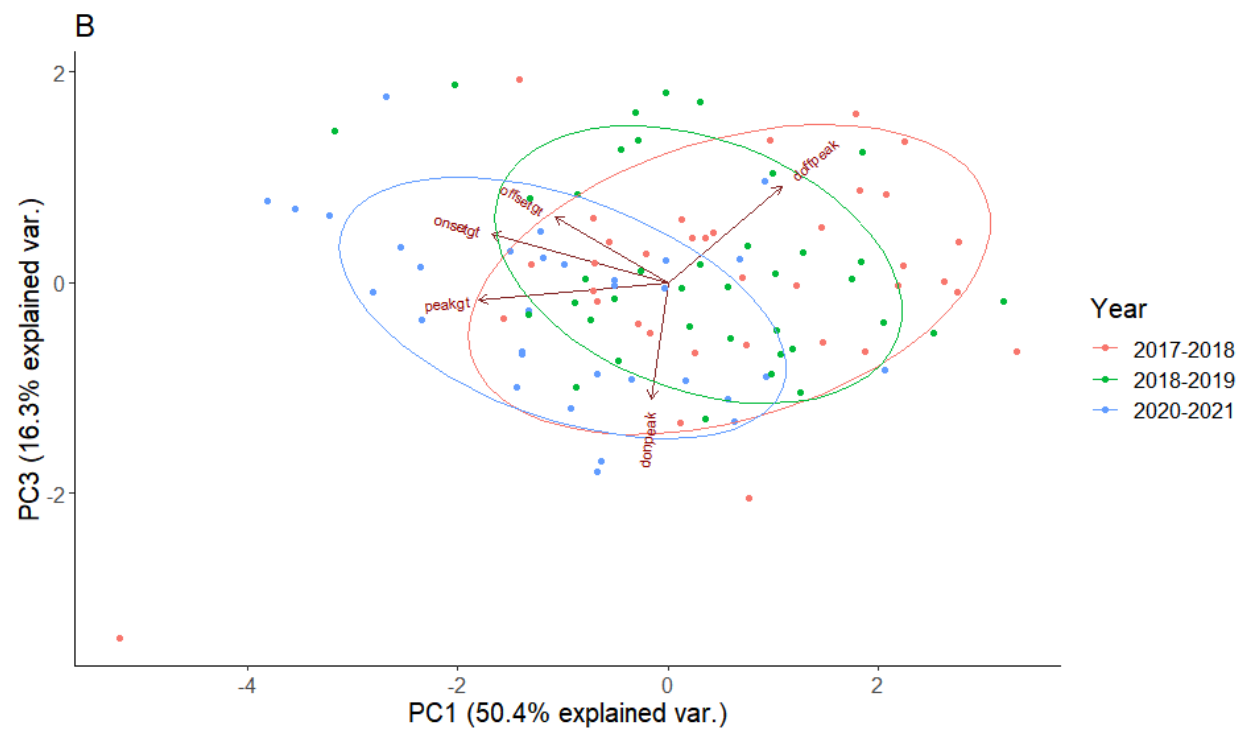| 2020-21	Yolo Region site 2	0	0	0	137.2	15.2	152.4	137.2	15.2	152.4
2020-21	Imperial Valley	221	221	442	27.9	0	27.9	248.9	221	469.9

Figure S1. Map of test locations in California.
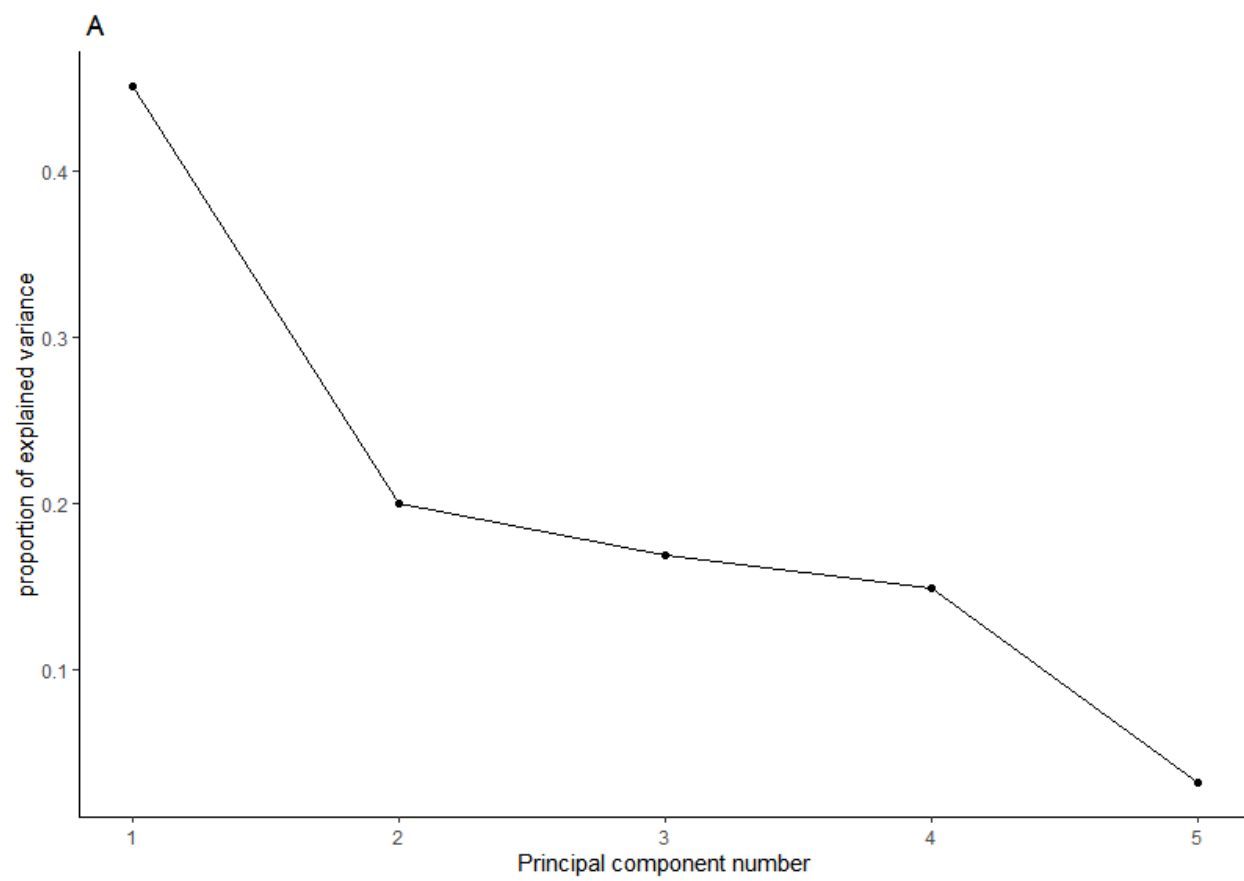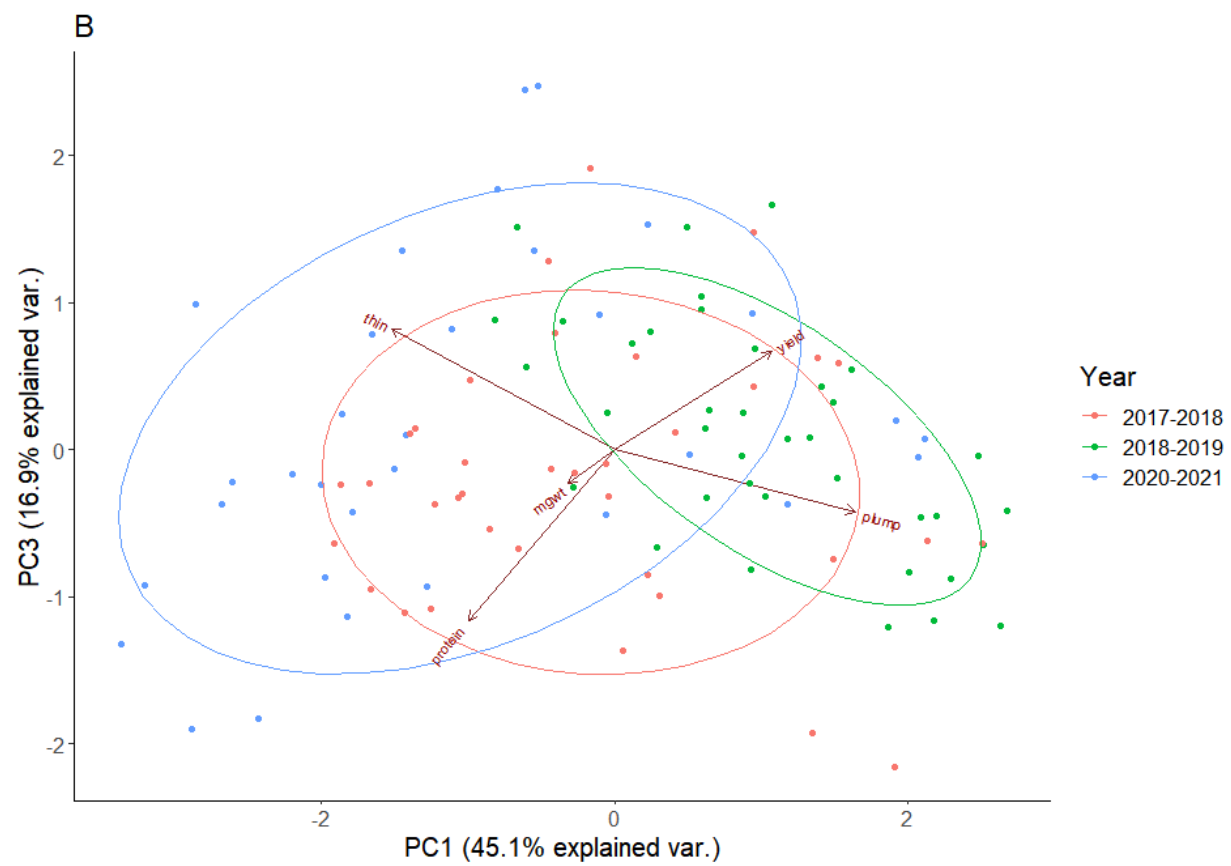
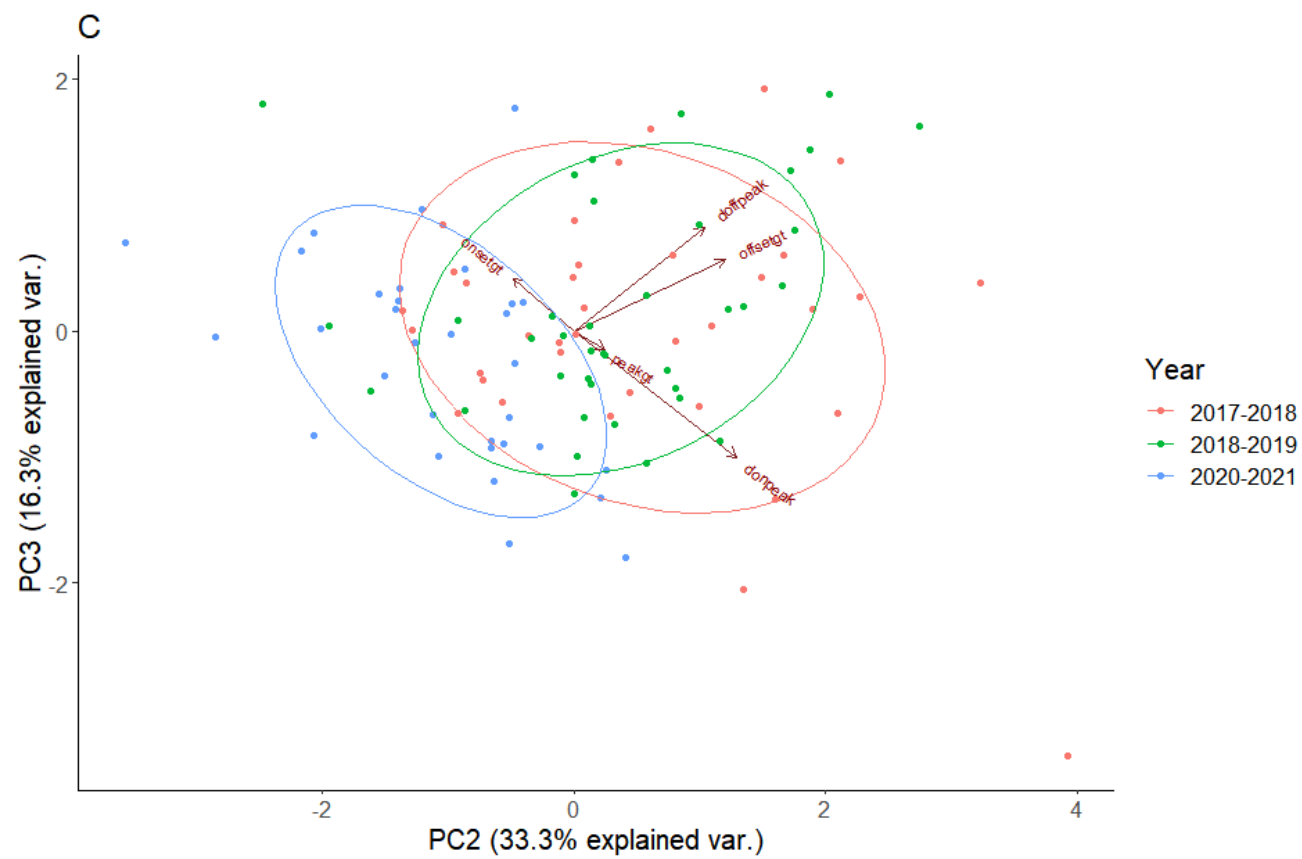Figure S2. Year based biplots of productivity and quality traits A) PCA scree plot; PCs B) 1 and 3; C) 2 and 3.
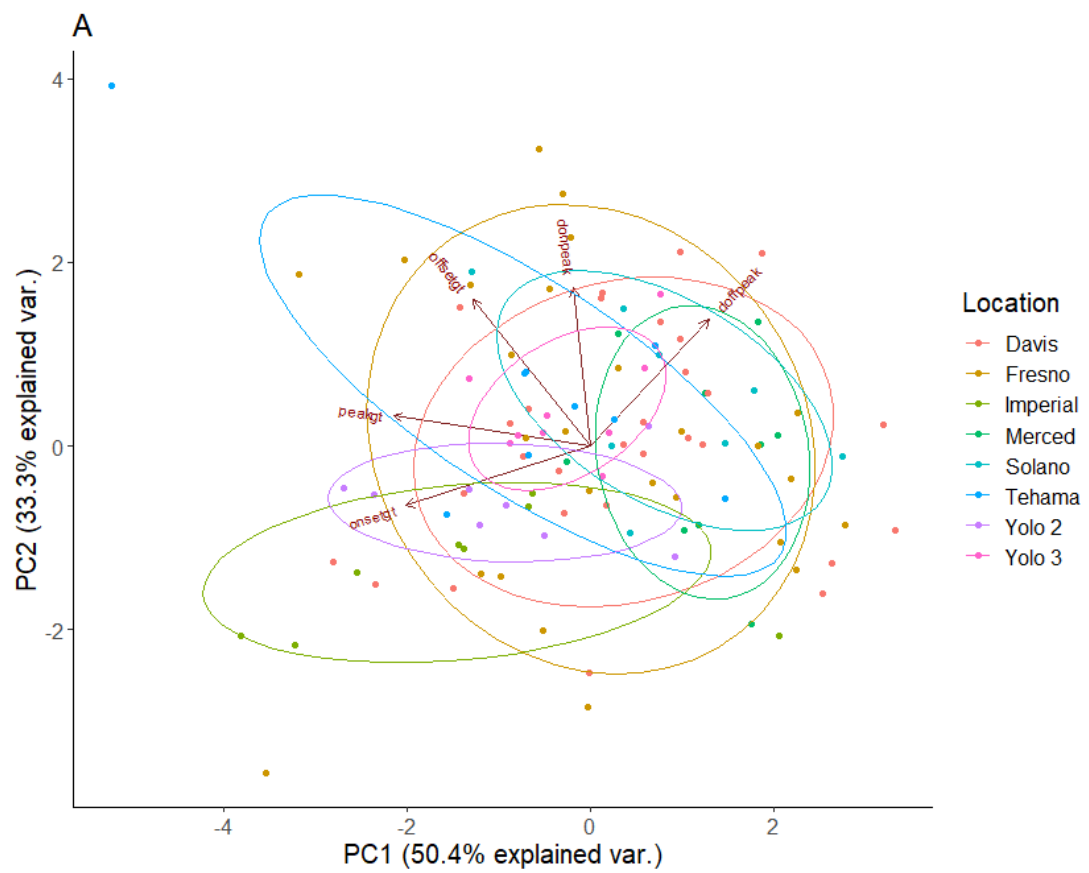
Figure S3. Year based biplots of starch GT traits A) PCA scree plot; PCs B) 1 and 3; C) 2 and 3.
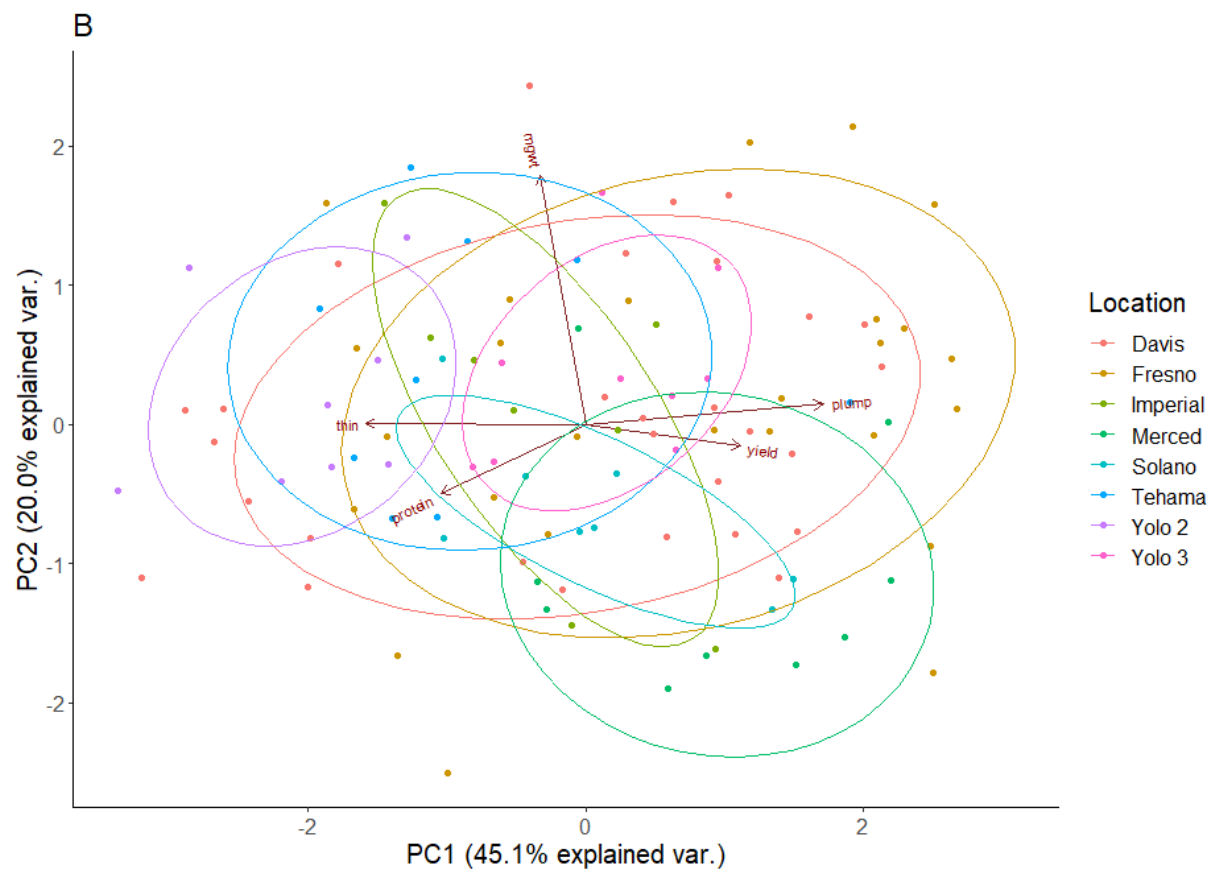
Figure S4. Location based biplots for A) PCs 1 and 2 of starch GT traits; B) PCs 1 and 2 of productivity and quality traits.