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Specific weight of barley grains is determined by traits affecting packing efficiency and by grain density

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Abstract

Background

Specific weight influences the market value of barley grain, and in malting barley a high specific weight is thought to result in an increased malt output. However, links between specific weight and malt output have not yet been established. We hypothesised that packing efficiency and grain density will each contribute to specific weight. These traits would have implications for the malting process, highlighting the need for understanding what grain traits contribute to specific weight, before we can predict its effect on malting performance and efficiency.

Results

We report that specific weight is a product of grain density and packing efficiency, in our study proportionally contributing 48.5% and 36.5% to variation in specific weight, respectively. We report that packing efficiency is determined by grain dimensions, and is negatively correlated with the sum of grain length and depth. Therefore shorter, thinner grains can result in an increased specific weight, which is likely to be detrimental for malting performance. We also demonstrate that among cultivars which have grains with contrasting size traits, the same specific weight can be achieved through differing grain densities.

Conclusions

Our results demonstrate that both grain dimensions and grain density must be considered jointly to optimise specific weight, and that the relationship between specific weight and malting performance and efficiency needs to be carefully considered with respect to how a high specific weight is achieved.

Keywords: *Hordeum vulgare,* Grain quality**,** Malting barley**,** Grain dimensions

1. Introduction

Specific weight (SW) is a measure of the weight of grain per unit volume and is used as a grain quality criterion for major cereals and oilseeds. Confusion can arise from the use of inconsistent terminology surrounding this criterion in the literature. 'Test weight', 'grain density', 'bushel weight', 'hectolitre mass, 'hectolitre weight' and 'bulk density' have all been used to describe this criterion. The traditional industry standard for measuring SW is using a chondrometer, which consists of two stacked cylinders separated by a sliding gate. The upper cylinder is filled with grain, the gate withdrawn and re-inserted once the grain has fallen. The grain in the lower cylinder of known volume is weighed and used to calculate SW in kilograms per hectolitre (kg hl^{-1}). Additional industry standards used to measure SW include a Dickey-John analyser or prediction using near-infrared spectroscopy [1].

In barley (*Hordeum vulgare*) SW influences the price of grain for both the feed and malting industries. Malting is the process of controlled grain germination in order to make the starch stored within the endosperm available for later enzymatic hydrolysis to maltose [2]. In the UK, spring barley is the main crop used for malting, as the grains have a high proportion of starchy endosperm and are therefore ideal for securing a good malt yield. The malt industry demands grain of a high SW, as it is assumed that a bulk of grain with high SW will contain a high proportion of endosperm biomass [3]. Grain 'plumpness' is one trait that is believed to positively contribute to SW and also benefit the malting process resulting in good extract levels due to higher levels of starch in the endosperm[4,5]. However a recent study showed that there is no significant correlation between starch content and SW in barley grains [6]. Grain bulks with a low SW incur penalties from industry and in extreme cases can even lead to rejections at a maltings. However correlations between barley SW and hot water extract, the main predictor of malt yield used in industry, have yet to be shown.

The very definition of SW indicates that it will be influenced by grain weight, and how well the grains pack into a volume. Indeed, dividing a sample's specific weight by grain density has previously been used to estimate the packing efficiency (PE) in cereal grains [7,8]. This relationship between SW, grain density and PE has not been applied to barley grains to the same extent as it has to oats and wheat. Determining that this relationship holds true among cultivars of spring barley would allow the examination of how each of the components, PE and density, contribute to SW differences among genotypes. This would be valuable information for barley breeders as SW is an important breeding target for malting barley. The ability to define SW by these two components will allow each one to be investigated individually not only to enhance our understanding of the formation of SW, but to assess their impact on malting performance.

It is clear there is a knowledge gap in identifying what attributes of spring barley grains influence SW. This needs to be addressed prior to investigating the effect of grain attributes on the malting process and product. In this study, we measured grain dimensions, weight, volume and two-dimensional area of 100 individual grains of nine cultivars to develop a detailed grain-level understanding of cultivars with a range of SWs. Grain density and PE were calculated and grain size manipulated to determine how these contribute to the SW of barley grains. Correlations among all measured grain traits were also examined to understand links among traits and between them and SW.

2. Methods

2.1 Grain samples

Nine spring barley malting cultivars from the Agriculture and Horticulture Development Board's (AHDB's) Recommended List (RL) 2016/17 were used in this study: KWS Irina, Octavia, Odyssey, Laureate, Origin, Concerto, Olympus, Propino and Sienna (https://cereals.ahdb.org.uk). These cultivars were chosen due to their phenotypic range in SW and varying levels of screenings, according to AHDB's RL 2016/17. The purpose of including multiple cultivars with a range of SWs was to extend the phenotypic variation in SW and its components, in order to better characterise relationships among SW and grain characteristics. All grain samples were grown in Docking, Norfolk under natural rainfall conditions during the 2016 season for the AHDB's RL crop trials. Prior to analysis, samples were cleaned by shaking over a 2.50 mm slotted sieve, with 19.05 mm long slots for 20 seconds. Grain retained by the sieve was used for analysis.

2.2 Specific weight

To achieve a detailed grain-level analysis of how differently shaped grains pack within a volume, and influence SW, it is necessary to have a scaled-down procedure for measuring SW which corresponds to the industry standard measurements, similar to that described by Gooding *et al*. (2003) [9]. Therefore, an accurate scaled-down method for measuring SW was developed in this study. Grain was poured from a height of 2 cm into a 25 ml measuring cylinder until it overflowed and superficial grains were removed by striking across the top of the cylinder with a straight edge. The total volume of the cylinder (39.16 ml) was obtained by weighing the amount of water required to fill the cylinder (Kern analytical balance PLJ 750-3N, accuracy ± 0.01 g). The weight of grain in the cylinder was divided by cylinder volume and multiplied by 100 to give an estimate of SW in kg hl^{-1}. The results from this scaled-down method were highly correlated with an industry standard measurement of SW in a trial ($r^2 = 0.84$, $P < 0.001$). This technique of estimating SW is similar to that described by Gooding *et al*. (2003) [9] and Walker and Panozzo (2011) [10].

2.3 Representative sampling

Grain samples (350 g) were sieved sequentially into the following size fractions using a stack of slotted 3.25, 3.00, 2.75 mm sieves, with 19.05 mm long slots: large (>3.25 mm), medium (3.25 to 3.00 mm), small (3.00 to 2.75 mm) and very small (<2.75 mm). The weight of grain in each fraction was recorded (Kern analytical balance PLJ 3500-2NM, accuracy ± 0.01 g) and where the fraction size was greater than 25 g SW was measured in triplicate using the scaled-down SW measurement described above. A 100 grain sample was taken from each fraction, and the mean grain weight from each fraction was used to estimate the total grain number in each size fraction and in the whole sample. A number of grains proportional to the total number of grains from each fraction were chosen at random, to give a 100-grain sample that was representative of the grain size distribution within the larger bulk sample.

2.4 Grain size parameters and image analysis

On the representatively sampled 100 grains from each of the nine cultivars the following measurements were taken. The grain dimensions length (L), width (W) and depth (D) were measured (see Supplementary Fig. S1) using a hand-held digital caliper (accuracy ± 0.01 mm). These dimensions were used to calculate grain sphericity which was calculated as the cube root of L × W × D divided by L [11]. This value was multiplied by 100 to give a percentage, with a value of 100% representing a sphere. The two-dimensional (2-D) area of grains was measured using ImageJ (National Institutes of Health, USA, https://imagej.nih.gov/ij/). All of these measures describe grain "size", which in this study refers solely to physical dimensions of the grain, whereas "weight" refers to mass. Individual grain area density is a measure of the mass per unit area (mg mm^{-2}), a combination of size and weight, and was calculated by dividing grain weight by 2-D area.

2.5 Packing efficiency and grain density

Grain volume and density were measured on the same 100-grains as above. Grain volume was measured by water displacement, with the weight of water displaced being equal to the volume of the grain (Archimedes' Principle). Grains were individually weighed using a Mettler AE 160 electronic balance (Mettler, Toledo, accuracy ± 0.0001 g) then submerged using a 0.5 mm x 25 mm hypodermic needle (BD Microlance) into a beaker of water using the same balance. Grain density (g cm^{-3}) was calculated by dividing the grain mass by grain volume. Packing efficiency was defined as the proportion of space occupied by the grain in the 25 ml cylinder above, and was calculated by multiplying mean grain volume by the mean grain number in the cylinder, divided by the cylinder volume. Mean grain number was calculated from three cylinder re-fills.

2.6 Data analysis

All data analysis was carried out using R software version 3.4.1 [12]. An analysis of variance ($\alpha = 0.05$) was done to determine whether the choice of different cultivars was successful in achieving significant differences in measured grain traits, thereby extending the phenotypic range within the analysed samples. Cultivar was found to be a significant factor in all grain traits apart from volume. Post-hoc Tukey's Honestly Significant Difference ($\alpha = 0.05$) tests were done to determine which cultivars were significantly different from each other to gain insight into whether differences in grain traits among samples corresponded with sample differences in SW. For sequential sieve analysis the effect of fraction size and cultivar among SW samples was analysed using a multiple linear model. Calculation of 95% confidence intervals using the 'emmeans' package[13] was used to compare the SW between grain fractions both within and between cultivars. The effect of the product of PE and grain density on SW among the three replicated samples measured was analysed using a simple linear regression. For this model the y-intercept was removed as it can be assumed that when SW is equal to zero the product of PE and grain density is also zero. A two-way ANOVA was done with SW as the dependent variable and PE and grain density as the two independent variables. To determine the relative contribution of both PE and density to the variance in SW the proportion of the sums of squares (SS) for each variable to total SS was calculated. Principal component analysis (PCA) was carried out using mean individual grain dimensions (L, W and D), plots of scores were created to investigate grain shape among the nine cultivars. The associations among all measured traits describing both individual grains and grain bulks were studied using a correlation matrix of Pearson correlation coefficients, which was produced using the 'corrplot' package[14].

3. Results

3.1 Grain traits

Grain traits were measured on 100 representatively sampled grains from each cultivar; the mean values and standard error of the mean for the 100-grain samples are presented in Table 1 for each cultivar as 'Individual Grain Analyses'. Significant differences in traits among grain samples were achieved in this case through use of cultivar selection within this 2016/17 field trial, providing a wide range of grain phenotypes with which to investigate performance of grain bulks. The 'Bulk Analysis' traits were measured on the larger bulk sample of each cultivar as supplied from AHDB, and the mean and standard deviation of these technical repeat measurements are presented in Table 1 to give a measure of variation within the bulk for these measurements. Cultivar samples are listed in order of descending bulk SW, from Sienna with the highest (69.40 kg hl^{-1}) to KWS Irina with the lowest (64.53 kg hl^{-1}). Among the grains sampled, Concerto had the lowest grain weight (47.49 mg) which was significantly lower than grains of Sienna ($P < 0.05$), Propino ($P < 0.05$) and Laureate ($P < 0.001$). Concerto also had the shortest (7.79 mm) and least wide (3.80 mm) grains, which were significantly shorter than grains from all other cultivars and less wide than Origin ($P < 0.0001$), Olympus ($P < 0.0001$), Laureate ($P < 0.01$) and Propino ($P < 0.05$). Grain volume and 2-D area were lowest in Concerto (37.85 mm^3, 21.71 mm^2), although its volume was not significantly smaller than any other cultivars its 2-D area was significantly smaller than Laureate ($P < 0.0001$), KWS Irina ($P < 0.0001$), Origin ($P < 0.001$) and Odyssey ($P < 0.05$). Sphericity was significantly higher in Concerto (57.62%) than all other cultivars. In terms of bulk analyses Concerto had the highest number of grains in the measuring cylinder (555.5). Laureate had the highest grain weight (52.45 mg) which was significantly higher than Octavia ($P < 0.05$), Olympus ($P < 0.01$) and Concerto ($P < 0.001$). Laureate also had the highest volume and density (40.37 mm^3, 1.31 g cm^{-3}), although its volume was not significantly larger than any other cultivars its density was greater than Octavia ($P < 0.01$), Concerto ($P < 0.01$), KWS Irina ($P < 0.001$) and Odyssey ($P < 0.0001$). In terms of bulk analyses Laureate had the lowest mean grain number in the cylinder (492.2) and packing efficiency (50.7%), compared to all other cultivars. Despite grains within the Laureate and Concerto samples having significantly different dimensions and weight, the SWs of 66.33 kg hl^{-1} and 66.84 kg hl^{-1} of each cultivar sample respectively, are very similar to one another. These results demonstrate that among grain bulks, the same SW can be achieved through different combinations of grain traits.

3.2 The effect of grain fraction size on specific weight

To examine how grain size correlates with specific weight among bulks, samples from each of the cultivars were sequentially sieved into different grain size fractions, creating a total of 25 samples with different grain sizes. Not all fractions were represented within each cultivar since not enough grain was retained of every size fraction for a SW estimate to be measured. Analysis of the SW of grain size fractions produced indicated signifcant differences between the largest and smallest fractions present for five out of the nine cultivar bulks (Fig. 1), these were: KWS Irina, Octavia, Laureate, Concerto and Propino. For these five cultivars, the smallest size fraction yielded grain with a higher SW than the largest fraction size. KWS Irina, Origin and Olympus only had the three smallest size fractions, whereas Octavia, Laureate, Concerto and Propino had the three largest size fractions. Both Odyssey and Sienna only had enough grain for estimates to be made on the middle two size fractions. This demonstrates that within these bulk samples, these two cultivars have a more uniform grain size than the other seven when grown in the conditions of this trial. This may vary when cultivars are grown under different environmental conditions during another season or location. Specific weight was not consistent for size fractions among samples from different cultivars. For example, the medium size fraction for Sienna which had a SW of 70.1 kg hl^{-1}, which was significantly greater than the medium size fractions of all other cultivars. These data demonstrate that grain size alone is insufficient to determine SW among bulks, and that density and packing efficiency of the grains must be taken into account.

3.3 Defining specific weight by its components: packing efficiency and grain density

Regression analysis showed a strong positive correlation between the product of PE and grain density with SW ($r^2 = 0.66$, $P < 0.01$) among the 100-grain samples from each cultivar. The output of the linear regression is shown by the solid black line and the equation SW = 0.988 × (PE × grain density) (Fig. 2). Seven of the nine cultivars appear close to the y=x line, shown by the dashed line, with four of these almost exactly on this line. This demonstrates that for the vast majority of cultivar samples used, the procedure used to estimate SW through PE and grain density was successful. Two cultivar samples however, KWS Irina and Sienna, are beneath the linear regression due to PE × grain density being larger than the SW. Through examining the mean grain weight of the 100-grain sample and mean weight of grains in the cylinder KWS Irina and Sienna had the greatest differences of +1.11 mg and +1.30 mg respectively (see Supplementary Table S1). An ANOVA showed that both PE and grain density had a statistically significant effect on SW at $P < 0.01$ (Table 2). Further analysis using the sum of squares to calculate the proportion of variation contributed by each component showed that PE contributed to 36.5% of the variability in SW, and grain density contributed 48.5%. The contribution of the residual error was small at 15.0% (Table 2).

3.4 The influence of grain dimensions on packing efficiency

Grain shape was further investigated through principal component analysis (PCA). The loadings and variance explained of the principal components (PCs) are reported in Supplementary Table S2. Principal component 1 (PC1) contributed 91.8% of the total variance, cultivars with a high score in PC1 tended to have shorter grains. Principal component 2 (PC2) contributed 5.3% to the total variance, cultivars with a high PC2 score have deeper grains. The relationship between grain length, width and depth and the PCs are shown in figure 3. A principal component biplot of PC1 against PC2 (Fig. 3) shows cultivars with longer grains have a lower PC1 score such as Laureate, Odyssey, KWS Irina and Origin. As cultivars increase in length from Concerto with the shortest grain length to Origin with the longest grain length, they have a higher PC1 score. Further separation occurs by PC2, cultivars with deep grains have a more positive PC2 such as Octavia, Laureate, Propino and Odyssey. Again, this analysis shows the difference in grain size between Laureate and Concerto, which occupy opposite sides of the plot. The plot separates cultivars according to their grain dimensions, which also corresponds to a diagonal gradient of grain number in the cylinder, because a greater number of small grains pack into the cylinder. Therefore Laureate is positioned in the far top left as it has the largest grains and hence fewest in the cylinder (492.2). The next diagonal portion of the plot is occupied by Origin, KWS Irina, Odyssey Octavia and Propino with similar grain numbers of 527.2, 520.3, 522.5, 522.3 and 523.0 respectively. The final diagonal portion in the bottom right of the plot has cultivars with the highest grain numbers Sienna (544.7), Olympus (549.5) and Concerto (555.5).

Grain number is one aspect of PE, therefore grain dimensions may help to partly explain PE but not the full extent of this component of SW.

3.5 Combined correlation analysis on grain parameters

The significance of correlations between measured traits was analysed, and a matrix of Pearson correlation coefficients (r) is given in Table 3. The significant correlation between sphericity and grain 2-D area ($r = -0.77$, $P < 0.01$) highlights that more spherically shaped grains have a reduced 2-D surface area. The negative correlation between grain number and length, ($r = -0.77$, $P < 0.05$) confirms the discovery in the previous PCA that fewer longer grains pack into a cylinder. This can also be related to grain volume, since grain number and volume negatively correlate ($r = -0.72$, $P < 0.05$). The negative correlation between the grain dimensions, length and depth with grain number was further explored in supplementary Fig. S2. The sum of grain length and depth correlates very strongly with grain number ($r = 0.90$, $P < 0.01$) (see Supplementary Fig. S2A) and with PE ($r = 0.75$, $P < 0.05$) (see Supplementary Fig. S2B). The sum of grain depth and length in this analysis strengthened the correlation between the dimensions and both grain number and PE than just length alone. Another strong positive correlation was observed between area density and SW ($r = 0.81$, $P < 0.05$). Area density summarises the weight of grain in a given area and SW is a measure of the weight of grain in a given volume, therefore the strong correlation between these variables was expected.

4. Discussion

How grain dimensions, weight, volume and PEs combine to determine the final SW within a grain bulk, or among cultivars, has previously not been established. Since SW is embedded in global grain trade as a measure of grain quality, an enhanced understanding of these traits is essential. Previous assumptions made that SW is a good predictor for the nutritional value of wheat have been upturned [15]. Therefore assumptions made about the value of SW for malting need to be investigated to ensure it is an effective measure of grain quality.

Studies on other cereal species which use SW as a measure of grain quality have used the equation SW = PE × grain density [8,16]. The current work demonstrated that this is also the case for barley grain, where the linear regression nearly mirrored the y=x line. The knowledge that barley SW can be defined by PE and grain density is an integral step towards enhancing our understanding of SW. Analysis of the relative contribution of each of these components to SW highlights that the contribution of one component does not vastly outweigh the other. Therefore both PE and grain density are the two defining contributors to SW and the grain traits that affect both of these components need to be analysed in turn.

In this study, grain traits of individual barley grains and also bulk level grain samples were analysed to investigate SW as a measure of grain quality. We have shown that observing just one grain trait or bulk character is not enough to understand SW. However, combining variables leads to a better understanding of SW and its components. This is highlighted by the non-significant relationships between: grain weight and SW; grain 2-D area and SW; and grain density and SW. However, for the combined variable 'area density', a strong and significant correlation is observed with SW. Therefore grain shape does not solely determine SW, nor does grain weight or density. Specific weight is influenced by a combination of all of the grain traits examined in this study. A multivariate approach therefore needs to be considered when analysing SW and its components.

The influence of grain dimensions on PE was investigated further through PCA. Here we demonstrated that grain dimensions length and depth strongly influence the number of grains in a vessel. The negative relationship between PE and these two grain dimensions is of borderline significance, which isn't improved by including grain width in the analysis. This highlights that grain dimensions as studied here in three planes (L, W and D) can't fully describe PE. What can be concluded is that cultivars with shorter, less deep grains pack more into a vessel and tend to have an increased PE, but other factors such as grain morphology could influence PE. In oat grains, Doehlert *et al*., (2006) [17] observed a strong negative correlation between length and SW this could partly be explained by the relationship between grain length and number in this study. Future grain morphological analysis will combine grain size and shape. The analysis of grain shape will involve quantifying shape, describing grains as more rounded or pointed through morphometrics.

Clarke *et al*. (2004) [18] reported a positive correlation between wheat grain size and SW, although in their study, "grain size" was a principal component vector encompassing grain mass alongside grain dimensions, area and perimeter. In our study, a higher grain size fraction negatively influenced SW in five out of the nine cultivars (Fig. 1), demonstrating that the effect of grain size fraction on SW is not uniform across cultivars. In the remaining four cultivars no significant effects on SW between the smallest and largest grain size fractions were found. The difference in results between these two studies is likely to be a result of the different methods of grain size manipulation. Clarke *et al*. (2004) [18] manipulated grain size by irrigation and nitrogen application, but we achieved this through sequential sieving. Sequential sieving influences size and may result in grain fractions of differing densities, but the effect of this is not the same as the environmental effect. Therefore it can be suggested that not only grain size influences SW, but also the environmental conditions or genotype leading to this size change. Other factors such as weathering, awn retention, grain shape and grain density affect SW, further demonstrating the potential environmental and genotypic influences on this trait [19].

When the same technique of sequential sieving was used with oat grains Doehlert *et al*., (2006) [20] found that smaller grain fractions resulted in increased SW, as found in the current study in five out of the nine cultivars. Doehlert *et al*. (2006) [20] observed grand means of size fraction SWs of numerous grain samples, so whether this effect is consistent among all cultivars used in their study is unknown. Grain size is a trait that has been suggested to affect malting and the results of this study provide a link between a factor that influences SW and also impacts upon malting [21,22]. In particular homogeneity of grain size is thought to be beneficial for malting to ensure uniform rates of water uptake by the grain, and consequential germination and endosperm modification.

Since PE is a major component of SW it is important to consider the potential influence of this on the malting process. It can be assumed that grain bulks with different PEs have an altered pore space distribution within the bulk of grains. Neethirajan *et al*. (2006) [23] showed that different pore space distributions within the bulk formed by cereals lead to an altered air flow through the bulk, in both the vertical and horizontal directions. This is likely to be extremely relevant to malting, where the first step in the process is steeping, which involves the soaking of grains in water. The barley grains imbibe water in this step increasing in moisture content and germination is initiated. Since PE will affect pore distribution, this could in turn influence the flow of water between grains. This will affect whether all grains in the bulk reach sufficient moisture content to germinate, impacting on steeping duration and efficiency. The same principles can be applied to kilning when hot air is passed through the malt, an irregular pore space distribution could lead to an unevenly kilned malt product.

The second major component of SW is grain density, the determinants of this were not investigated in this study. However, it is hypothesised that grain density, unlike PE is primarily influenced by grain composition and internal structure rather than morphological features of the grain[10]. Aspects of grain composition that could influence density are: starch content, protein content, starch granule ratios, ratios of amylose and amylopectin, ratios of the different grain tissues and the internal packing of these within the grain[24]. If grain density is positively influenced by a compositional aspect which is beneficial for malt quality, for example a high starch content, this would reinforce the value of SW as a grain quality measure. However, if grain density is increased by factors associated with a poor malt, for example a high protein content this would bring the value of this under question.

5. Conclusions

This study uncovers the contribution of the components PE and grain density to SW, and examines grain traits influencing these. When breeders target SW, this needs to be done through the correct balance of density and PE relevant to the end-use. Knowledge of this is important so the malting industry can understand exactly what the effect of differing SWs and their components are likely to have upon the malting process. The work gives insight as to why grain bulks with similar SWs and hence similar market value grain could lead to different malting efficiencies, via altered PEs due to grain size. Therefore SW alone may not be a comprehensive standalone measure of grain quality for the malting industry.

6. Acknowledgements

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Table 1

Measured[a] grain traits for the nine spring barley cultivars[b] examined.

	Cultivar								
	Sienna	Propino	Olympus	Concerto	Origin	Laureate	Odyssey	Octavia	KWS Irina
Individual Grain Analysis									
Weight (mg)	51.20 ± 0.79 ab	50.97 ± 0.79 ab	48.32 ± 0.75 bc	47.49 ± 0.78 c	49.36 ± 0.72 abc	52.45 ± 0.81 a	50.01 ± 0.73 abc	48.61 ± 0.85 bc	49.67 ± 0.75 abc
Depth (mm)	2.98 ± 0.02 bc	3.06 ± 0.02 a	2.91 ± 0.02 d	3.03 ± 0.02 ab	2.88 ± 0.02 d	3.03 ± 0.02 ab	2.95 ± 0.02 cd	3.01 ± 0.02 abc	2.91 ± 0.01 d
Length (mm)	8.12 ± 0.06 d	8.22 ± 0.06 cd	8.22 ± 0.06 bcd	7.79 ± 0.07 e	8.56 ± 0.06 a	8.53 ± 0.06 a	8.48 ± 0.05 ab	8.33 ± 0.07 abcd	8.45 ± 0.06 abc
Width (mm)	3.82 ± 0.02 cd	3.90 ± 0.02 abc	3.94 ± 0.02 a	3.80 ± 0.02 d	3.95 ± 0.02 a	3.93 ± 0.02 ab	3.85 ± 0.02 bcd	3.80 ± 0.02 d	3.89 ± 0.02 abcd
Volume (mm^3)	39.61 ± 0.65 a	39.61 ± 0.63 a	38.01 ± 0.62 a	37.85 ± 0.70 a	38.71 ± 0.57 a	40.37 ± 0.70 a	40.17 ± 0.57 a	38.39 ± 0.66 a	39.59 ± 0.66 a
Density ($g\ cm^{-3}$)	1.30 ± 0.01 ab	1.29 ± 0.01 abc	1.27 ± 0.01 abcd	1.26 ± 0.01 cd	1.28 ± 0.01 abcd	1.31 ± 0.01 a	1.25 ± 0.01 d	1.27 ± 0.01 bcd	1.26 ± 0.01 cd
2-D Area (mm^2)	22.26 ± 0.25 cd	22.53 ± 0.26 bcd	22.72 ± 0.27 bcd	21.71 ± 0.28 d	23.37 ± 0.24 ab	24.02 ± 0.25 a	22.94 ± 0.22 abc	22.38 ± 0.26 bcd	23.88 ± 0.26 a
Sphericity (%)	55.77 ± 0.20 bc	56.14 ± 0.21 b	55.44 ± 0.22 bcd	57.62 ± 0.27 a	53.81 ± 0.24 e	54.77 ± 0.20 def	54.07 ± 0.21 ef	54.97 ± 0.28 cde	54.16 ± 0.19 f
Area Density ($mg\ mm^{-2}$)	2.29 ± 0.02 a	2.25 ± 0.02 ab	2.12 ± 0.02 cd	2.18 ± 0.02 bc	2.11 ± 0.02 cd	2.17 ± 0.02 c	2.17 ± 0.02 c	2.16 ± 0.02 c	2.07 ± 0.02 d
Bulk analysis									
Grain Number	544.67 ± 2.08	523.00 ± 4.36	549.50 ± 3.46	555.50 ± 5.63	527.17 ± 3.33	492.17 ± 4.16	522.50 ± 8.79	522.33 ± 0.58	520.33 ± 4.54
PE (%)	55.09 ± 0.21	52.90 ± 0.44	53.34 ± 0.34	53.69 ± 0.54	52.11 ± 0.33	50.73 ± 0.43	53.60 ± 0.90	51.20 ± 0.06	52.60 ± 0.46
SW ($kg\ hl^{-1}$)	69.40 ± 0.38	68.05 ± 0.25	66.95 ± 0.28	66.84 ± 0.38	66.53 ± 0.37	66.33 ± 0.69	65.93 ± 0.24	65.53 ± 0.55	64.53 ± 0.67

[a]Individual grain analysis values are expressed as mean ± standard error of the mean and bulk analyses expressed as ± standard deviation.

[b]Cultivars which do not share a letter for each of the measured traits are significantly different from one another.

Table 2

ANOVA table for specific weight showing the proportional contribution[a] of packing efficiency and density to SW.

Source of variation	df	Sum of squares	Mean square	*F*-value	*P*-value	Contribution (%)
Packing efficiency	1	5.85	5.85	14.60	0.0088	36.48
Density	1	7.78	7.78	19.42	0.0045	48.52
Residuals	6	2.40	0.40			14.99
Total	8	16.03				

[a]Calculated as a percentage of the sum of squares for each variable

Table 3
Correlation matrix[a] of Pearson correlation coefficients (r) for grain dimensions, shape parameters and components of SW.

	Weight (mg)	Depth (mm)	Length (mm)	Width (mm)	Volume (mm^3)	Density (g cm^{-3})	2-D Area (mm^2)	Sphericity (%)	Grain Number	Area Density (mg mm^{-2})	SW (kg hl^{-1})	PE (%)
Weight (mg)	1	0.26	0.46	0.28	0.89**	-	0.51	-0.20	-0.69	-	0.30	-0.16
Depth (mm)		1	-0.47	-0.47	0.13	0.36	-0.41	-	-0.15	0.68	0.31	-0.11
Length (mm)			1	0.58	0.56	0.02	0.85***	-	-0.77*	-0.44	-0.46	-0.57
Width (mm)				1	0.16	0.31	0.68*	-	-0.35	-0.44	-0.06	-0.36
Volume (mm^3)					1	-	0.58	-0.45	-0.72*	0.27	0.02	-
Density (g cm^{-3})						1	0.16	0.17	-0.28	0.50	0.59	-0.15
2-D Area (mm^2)							1	-0.77**	-0.77*	-	-0.50	-0.57
Sphericity (%)								1	0.59	0.52	0.50	0.40
Grain Number									1	0.13	0.40	-
Area Density (mg mm^{-2})										1	0.81*	0.45
SW (kg hl^{-1})											1	0.59
PE (%)												1

[a]The symbol "-" indicates that one variable was used to calculate the other, therefore no correlation was calculated.

"***", "**", "*" were significant at P < 0.001, P < 0.01 and P < 0.05 respectively.

Figure captions

Fig. 1. Specific weight measured on four size fractions of nine spring barley cultivars. Size fractions are the following: very small (2.50 to 2.75 mm), small (2.75 to 3.00 mm), medium (3.00 to 3.25 mm) and large (> 3.25 mm). Cultivars are ordered from the lowest mean SW from KWS Irina to the highest mean SW, Sienna. When fractions share a letter the SWs are not significantly different from one another and when a letter is not shared the fractions are significantly different from one another, $P < 0.05$. Bars are the standard error of the means.

Fig. 2. The SW of nine barley cultivars plotted against the product of PE and grain density. The linear regression is shown by the solid black line, whereas the dashed line indicates the y=x relationship.

Fig. 3. Biplot of the principal component analysis of grain shape parameters of nine spring malting barley cultivars. Grain dimensions used in this analysis: L, length; W, width and D, depth. Arrows originating at the centre of the biplot represent the loadings of grain dimensions, with the length of these arrows corresponding to the relative importance of each dimension in each axis. Example grain shapes (not to scale) are shown on the plot to indicate which grain shapes have high or low scores in each of the principal components. Loadings for each grain shape parameter are included in a table beneath the biplot.

Supplementary Fig. S1. Anatomical diagram of a barley grain, indicating the orientation of dimensions measured in this study.

Supplementary Fig. S2. Linear regression plots of the sum of grain length and depth correlated with

(A) grain number ($r^2 = 0.81$, $P < 0.01$) and (B) packing efficiency ($r^2 = 0.44$, $P = 0.05$), for the nine cultivars.

Fig. 1

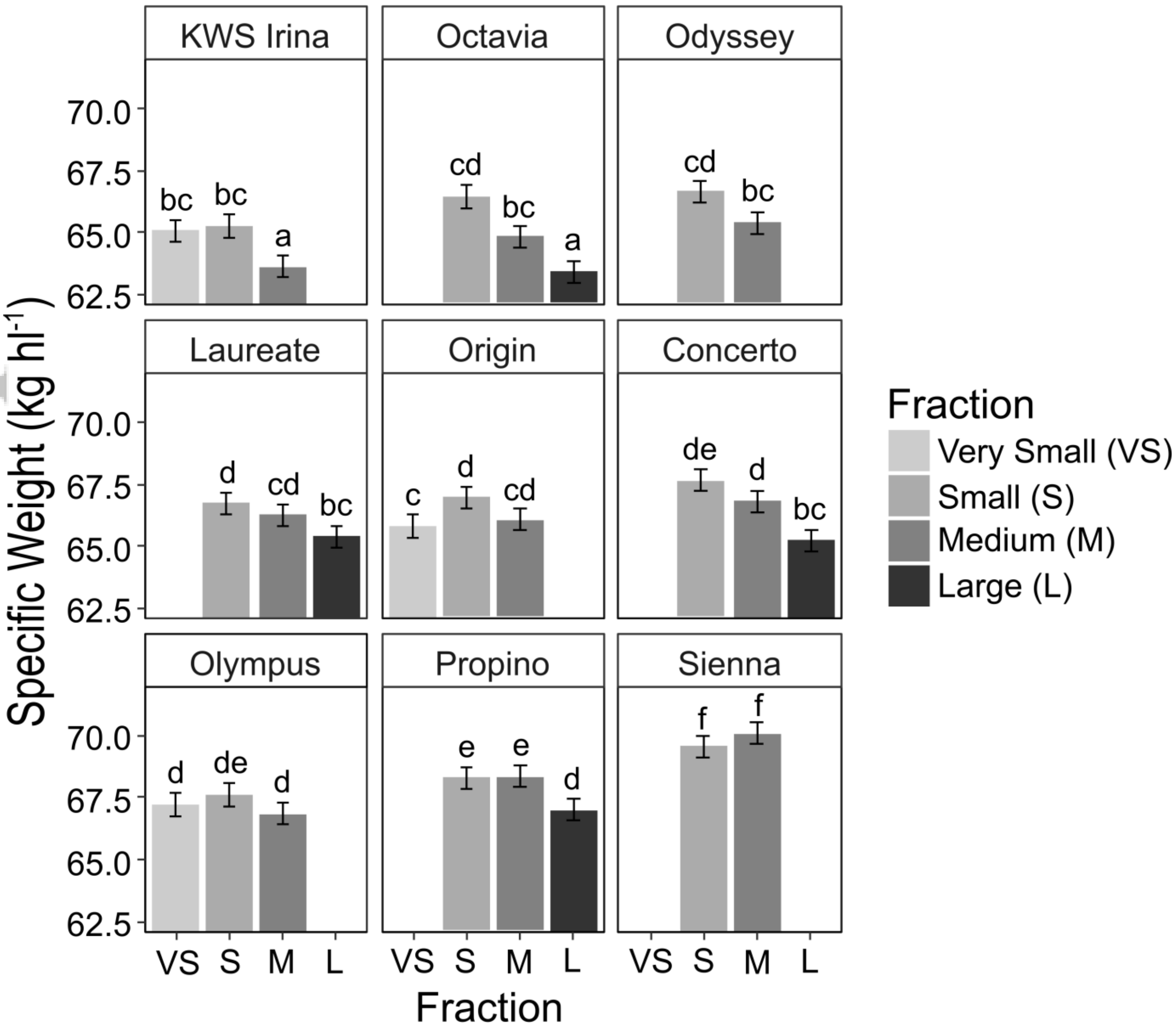


Fig. 2

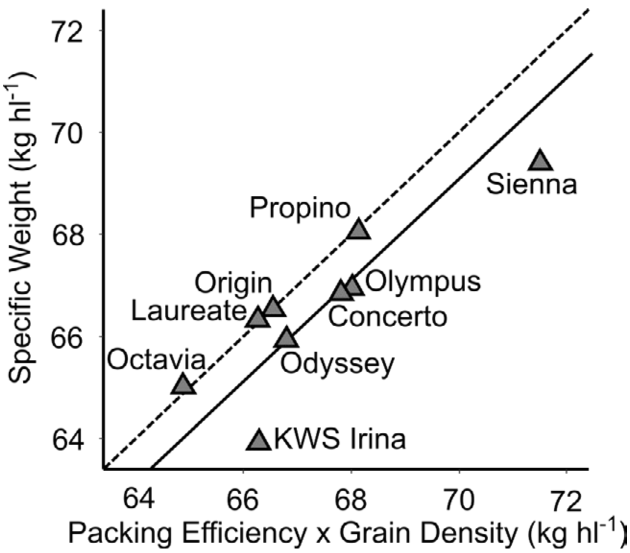
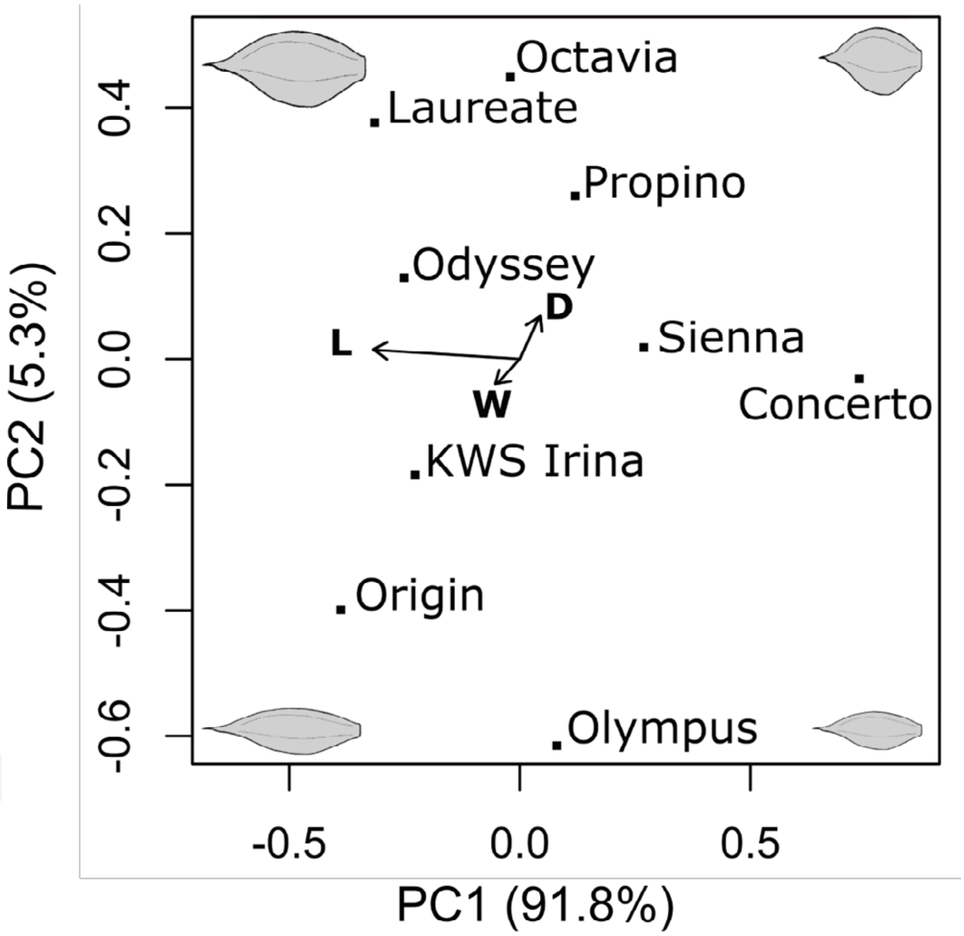

72
70
68
66
64
Specific Weight (kg hl^{-1})
Propino
Sienna
Origin
Olympus
Laureate
Concerto
Octavia
Odyssey
KWS Irina
64
66
68
70
72
Packing Efficiency x Grain Density (kg hl^{-1})

Fig. 3



Dimension	PC1	PC2
Length (L)	-0.981	0.185
Depth (D)	0.130	0.185
Width (W)	-0.146	-0.485