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The detection of QTLs in barley associated with endosperm hardness, grain density, grain size and malting quality using rapid phenotyping tools

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Abstract Using a barley mapping population, 'Vlamingh' × 'Buloke' (V × B), whole grain analyses were undertaken for physical seed traits and malting quality. Grain density and size were predicted by digital image analysis (DIA), while malt extract and protein content were predicted using near infrared (NIR) analysis. Validation of DIA and NIR algorithms confirmed that data for QTL analysis was highly correlated ($R^2 > 0.82$), with high RPD values (the ratio of the standard error of prediction to the standard deviation, 2.31–9.06). Endosperm hardness was measured on this mapping population using the single kernel characterisation system. Grain density and endosperm hardness were significantly inter-correlated in all three environments ($r > 0.22$, $P < 0.001$); however, other grain components were found to interact with the traits. QTL for these traits were also found on different genomic regions, for example, grain density QTLs were found on chromosomes 2H and 6H, whereas endosperm hardness QTLs were found on 1H, 5H, and 7H. In this study, the majority of the genomic regions associated with grain texture were also coincident with QTLs for grain size, yield, flowering date and/or plant development genes. This study highlights the complexity of genomic regions associated with the variation of endosperm hardness and grain density, and their relationships with grain size traits, agronomic-related traits, and plant development loci.

Introduction

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world and its grain is mainly used for animal feed, malting and brewing. Grain texture is an important end-use quality parameter, associated with the malting and processing quality (Holopainen et al. 2005; Mayolle et al. 2012; Psota et al. 2007; Allison 1986; Walker and Panozzo 2011). Two components of grain texture are endosperm hardness and grain density.

Endosperm hardness is a physicochemical component of grain texture; barley with a harder endosperm requires more energy to grind (Camm et al. 1990; Psota et al. 2007) and is associated with higher vitreosity (Mayolle et al. 2012; Holopainen et al. 2005), slower water uptake (Mayolle et al. 2012; Gamlath et al. 2008), higher protein content (Camm et al. 1990; Walker and Panozzo 2011), and denser grain (Walker and Panozzo 2011). Barley with softer endosperm contains lower β-glucans (Gamlath et al. 2008; Psota et al. 2007), lower content of non-starch polysaccharides (Gamlath et al. 2008), modifies faster in the malting process with higher malt extract (Psota et al. 2007; Allison 1986), and has brighter grain (Nair et al. 2010). Grain density is a measure of the compactness of a seed. Grain with a lower density has a mealy endosperm that contains loosely packed starch granules in a

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discontinuous protein matrix interspersed with vacuous spaces (Nair et al. 2010, 2011; Psota et al. 2007; Briggs et al. 2001; Chang 1988; Fang and Campbell 2000). Physical traits, such as grain weight and size, are used routinely to classify the harvest quality of barley. Large and plump grains are thought to be related to even water uptake and modification, and higher extractable sugar content (Li et al. 2008; Agu et al. 2007). The relationship among endosperm hardness, grain density, grain size, malting and processing quality remains a focus of research.

Plant developmental genes govern the genetic expression of grain size and weight traits (Coventry et al. 2003; Ayoub et al. 2002; Bingham et al. 2007). These grain traits are affected by grain yield, assimilate (source) availability, number of grains per m^2 (sink), flowering date, carbohydrate accumulation and deposition, and the rate and duration of grain filling (Coventry et al. 2003; Hayes et al. 2003). The formation and structure of the starch and protein matrix in the endosperm during grain development also contribute to the variation in texture of barley grain (Brennan et al. 1996). The link among plant development, grain yield, grain size, malting quality and grain texture remains unclear. Therefore, to gain an understanding of the genomic control of grain texture, traits such as grain size, weight, volume, yield, and plant flowering date should also be considered.

Several phenotyping tools have been used to assess grain quality, structure and shape. Three of these tools, namely single kernel characterisation system (SKCS), digital image analysis (DIA), and near infrared (NIR) spectroscopy, can assess multiple traits simultaneously on a small grain sample. The SKCS is used to determine endosperm hardness and can measure the variation within cereal mapping populations (Wang et al. 2008; Walker et al. 2011; Beecher et al. 2002; Turuspekov et al. 2008; Morris et al. 1999). DIA is an emerging non-destructive tool used to measure the variation in appearance and grain shape by recording individual grain images (Walker and Panozzo 2012; Walker et al. 2009; Ayoub et al. 2002; Edney et al. 2005; Williams et al. 2012). NIR spectroscopy is a non-destructive, rapid tool used to assess a range of traits including grain protein content (GPC) and hardness (Walker et al. 2011; Fox et al. 2007; Beecher et al. 2002).

Endosperm hardness, as measured by the SKCS, is calculated by the force and time taken to crush a grain, the moisture content, and the grain weight (Walker et al. 2011; Pearson et al. 2007). QTLs associated with SKCS hardness have been identified at the *Ha* locus on chromosome arm 5HS, and on chromosomes 1H, 2H, 4H, 6H and 7H (Beecher et al. 2002; Walker et al. 2011; Swanston 1995; Fox et al. 2007).

Grain density, as opposed to the bulk density, is a grain texture trait of little research focus in barley due to

difficulty in measuring rapidly. Currently, displacement methods are used to measure the grain volume so that grain density can be calculated; however, these methods are labour intensive and impractical when measuring hundreds of samples (Walker and Panozzo 2011). Alternatively, grain density on individual kernels has been estimated using grain volume measurements from 2-D digital images with moderate success ($r = 0.63$, $P < 0.01$) (Walker and Panozzo 2012). However, a higher accuracy in calculating grain density and grain volume, potentially achieved through 3-D imaging methods, is required for mapping and detection of QTL associated with grain density.

Two-dimensional DIA has also been used to derive grain size and shape data on individual barley grains (Walker and Panozzo 2012; Ayoub et al. 2002; Nielsen 2003). Data from 2-D images can be used to calculate grain length and major-width (Walker and Panozzo 2012; Sýkorová et al. 2009), however, the third dimension (minor-width) was not measured. The minor-width is an important trait for the malting industry, impacting on how barley is graded. Industry reference methods, using nested slotted sieves, sort the grain into size groups and the minor-width determines whether the grain will fall through or remain on top of the sieves (Black et al. 2008). QTLs specifically associated with DIA of the grain minor-width, similar to grain density, are yet to be reported in barley.

2-D DIA revealed QTL for grain size and shape on chromosomes 2H (near the *Vrs-1* locus) (Komatsuda et al. 2007) and 4H (near *int-c*) (Ramsay et al. 2011) in a two-row/six-row mapping population (Ayoub et al. 2002). Both QTLs were previously associated with grain plumpness and weight (Marquez-Cedillo et al. 2000, 2001). Subsequently, grain plumpness and weight QTLs were detected on all seven chromosomes (Mather et al. 1997; Walker et al. 2011; Moody et al. 2009; Coventry et al. 2003). These regions are thought to be associated with plant developmental genes, such as the vernalisation loci (Karsai et al. 2008; Szűcs et al. 2007), spike row number (Karsai et al. 2008), photoperiod response (Karsai et al. 2008), intermedium spike traits (Ramsay et al. 2011; Komatsuda and Mano 2002), and phytochromes involved with regulating flowering time (Szűcs et al. 2006). DIA has the potential to reveal QTLs associated with multiple grain size traits such as the dimensions of the grain, volume and density within mapping populations so as to build on and dissect current genomic knowledge.

The industry reference method for determining malt extract, a key malting quality trait, involves a 6- to 8-day controlled germination and kilning process, measuring the fermentable sugars in the malt (EBC-4.5.1 1998; IOB 1991). The majority of malt extract QTLs, identified on chromosome 1H and chromosome arms 2HS, 3HL, 4HS, 5HL and 7HL, have been based on industry reference

methods (Mather et al. 1997; Panozzo et al. 2007; Thomas et al. 1996; Gao et al. 2004). NIR-based screening was introduced to assist in the rapid selection of malting quality traits, such as GPC and malt extract, in barley in early generations (Bezant et al. 1997; Black and Panozzo 2002). For this, pre-cursors in the spectra measured on unmalted barley were correlated to malt extract values. Bezant et al. (1997) used NIR-predicted malt extract on ground barley and determined genomic regions associated with this trait. A major benefit of identifying malt extract-related QTLs using NIR-predicted values would be a greater understanding of which genomic regions are associated with grain texture and malting quality. However, rigorous validation against standard methods would be required to ensure accuracy of the calibration (Panozzo et al. 2007).

The goals of this study were to: (1) develop non-destructive, accurate, rapid tools for measuring grain texture, grain size and malt-quality related traits; (2) identify QTLs for grain texture, malt quality, grain size, grain yield and flowering date using a doubled-haploid (DH) population of 289 lines genotyped with 1,536 SNPs; (3) integrate published genes and QTL data related to plant development and malting quality along the DH population map based on locations in the barley consensus map; and (4) determine if the QTLs associated with the assessed traits were co-located and/or align with the published genes integrated along the map. The results of this study add to the existing knowledge and justification of potential regions for targeted selection of barley grain texture for breeding purposes.

Materials and methods

Plant materials

Two-hundred and eighty-nine DH lines (DHLs) developed from a cross between the barley varieties Vlamingh and Buloke were used in this study. Vlamingh and Buloke are two-row commercial malting varieties with similar malting quality characteristics and phenology, but that contrast in pedigree, grain hardness, grain density and grain plumpness. Buloke is more widely adapted in terms of disease resistance and yield potential, and Vlamingh has superior grain plumpness. The DH population was grown in non-replicated trials with grid controls in three environments over a 1-year period (2007) under rainfed conditions at Lake Bolac and Horsham, and under irrigated conditions at Horsham.

SNP genotyping and linkage mapping

Genomic DNA was extracted from barley plants according to the method described by Cane et al. (2004). The 1,536

SNPs of the Illumina barley oligo pool assay (BOPA1) (Close et al. 2009) were used to genotype the 'Vlamingh' × 'Buloke' (V × B) population (Close et al. 2009; Hyten et al. 2008). SNP data curation and genetic map construction were as described by Muñoz-Amatriaín et al. (2011). Briefly, monomorphic and low-quality markers as well as problematic and potential duplicated individuals were removed from the dataset. The resultant 440 SNPs and 289 DHLs were used for linkage map construction, using the software tool MSTmap (Wu et al. 2008), with a cut-off P value of 0.000001, a maximum distance between markers of 15.0 cM, no estimation before clustering, the COUNT objective function, and the Kosambi distance function.

Phenotypic measurements

Grain yields (t/ha) from harvest were recorded at the three sites (Moody et al. 2009). Flowering dates (the Julian Day, JD, where 50 % of heads were at Zadok growth stage 51) were also recorded on two sites of the three sites, Horsham (rainfed) and Horsham (irrigated) (Moody et al. 2009).

Endosperm hardness, grain density, malt extract, GPC, grain weight, grain volume, grain length, major-width and minor-width were measured on a 200-g grain subsample of each plot using the SKCS, image analysis and NIR techniques.

Endosperm hardness was measured by an SKCS 4100, Perten Instruments, Springfield, USA (Walker et al. 2011).

Malting quality traits, GPC and malt extract, were predicted on whole grain barley using a NIRSystems XDS (Foss Pacific Pty Ltd, Denmark), as described by Black and Panozzo (2002), Panozzo et al. (2007) and Walker et al. (2011), and both traits were expressed as a percent dry basis.

The grain weight (mg) was measured as the mass of 500 individual grains divided by 500.

The whole grain samples were analysed through a 3-D digital image analyser (Foss Pacific Pty Ltd, Denmark). Matlab (version 7.7.0.471, R2011b) with the image processing and statistical toolbox was used to process the Portable Network Graphics (PNG) images and determine grain size and volume. Any images containing foreign material, cracked and split grains were filtered from the analyses as described by Walker et al. (2009) and Walker and Panozzo (2012). Grain length and major-width (widest width section of the grain) were estimated from pixel lengths of the collected 2-D PNG images (Walker and Panozzo 2012). The minor-width (thinnest width section of the grain and the third dimension) was obtained from the laser cross section of the same grain. The volume was calculated using the summation of cylindrical segments along the length of the 2-D grain image and the
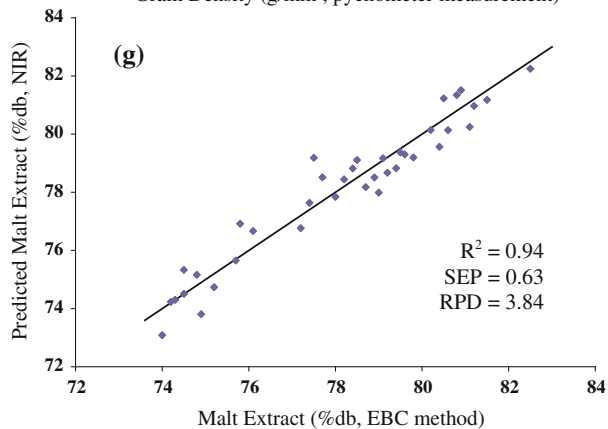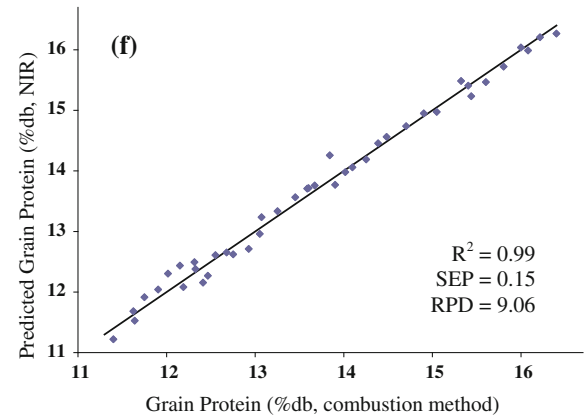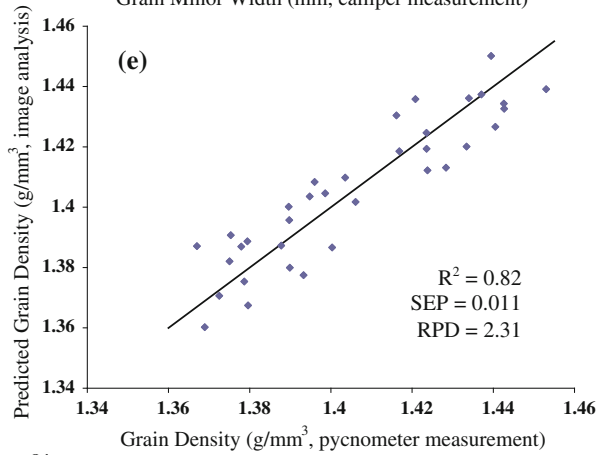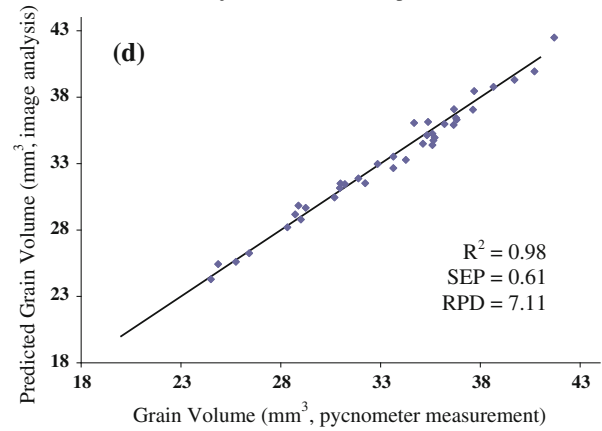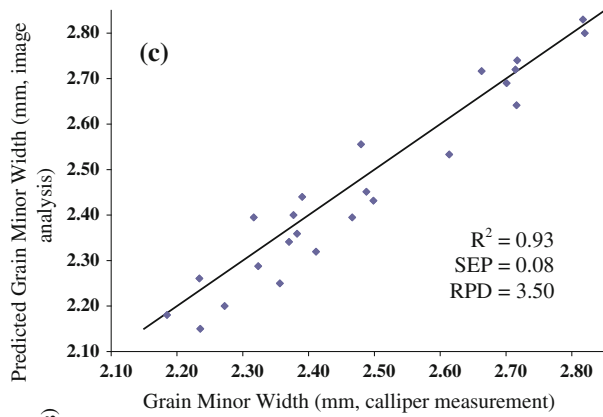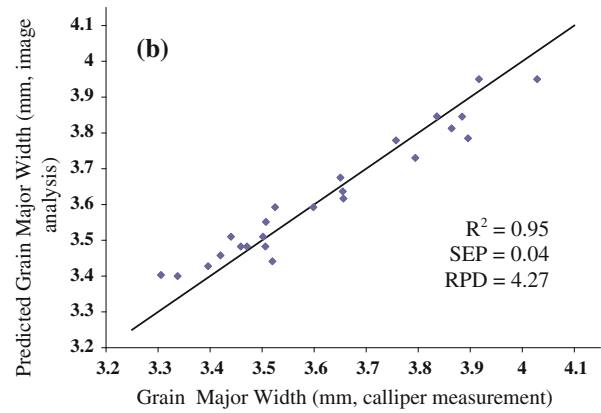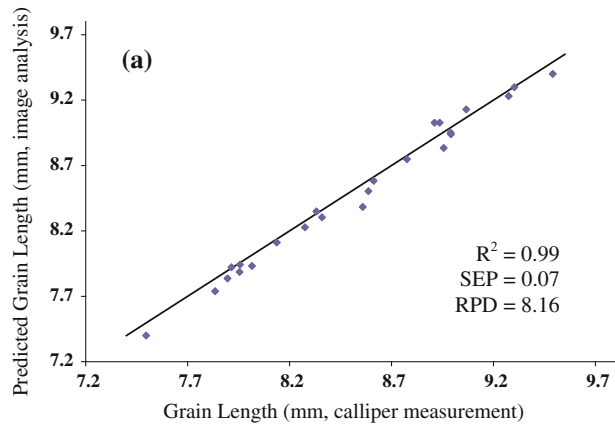
◄**Fig. 1** Scatter plots of the validation data **a** measured grain length plotted against the DIA-predicted values, **b** measured grain major-width plotted against the DIA-predicted values, **c** measured grain minor-width plotted against the DIA-predicted values, **d** gas displacement grain volume plotted against the DIA-predicted values, **e** grain densities calculated from gas displacement volume plotted against the DIA-predicted values, **f** reference grain protein content plotted against the NIR-predicted values, **g** reference malt extract content plotted against the NIR-predicted values, with a 1:1 line of best fit (*solid line*)

corresponding 3-D using laser cross section of the grain, as proposed by Walker and Panozzo (2012). This geometric approach was modified using the minor-width measurements of the laser cross section of the grain instead of the assumptions made from the correlation between the major and minor-width.

The grain density (mg/mm^3) of a sample was calculated by dividing the grain weight (mg) by the grain volume (mm^3) as measured by DIA.

Validation of quality traits

Three subsets of samples from each of the three trial environments were used for validation purposes. One subset, consisting of 25 samples, was assessed using callipers for grain length, grain major-width and grain minor-width (Walker and Panozzo 2012); a second subset, consisting of 38 samples, was measured for grain volume, and grain density using gas displacement (Walker and Panozzo 2011); GPC was measured on a second subset, consisting of 42 samples, using the Dumas combustion nitrogen procedure (Panozzo et al. 2007); and the third subset, consisting of 38 samples, was malted and the malt extract measured using a modified EBC method (Panozzo et al. 2007).

Three statistical methods were used to evaluate the accuracy of predicting traits using NIR or DIA; the coefficient of determination (R^2), the standard error of prediction (SEP), and the RPD, the ratio of the SEP to the standard deviation (SD) of the reference data (Williams and Norris 2001; Black and Panozzo 2004; Natsuga and Kawamura 2006). The RPD is a descriptive statistic used to assess the predictive accuracy of a technique, such as NIR, where the higher the value, the lower is the signal to noise ratio in the data set compared to reference methods. Ranges of RPD values have been used to evaluate NIR for various applications, from moderate accuracy for early generation breeding screening to high accuracy for industrial process and quality control (Williams and Norris 2001). Four RPD ranges defined by Natsuga and Kawamura (2006) were adopted by this study. A reported RPD value of less than 2.30 indicated poor accuracy in the validation set, and is not recommended for use in

genomic studies; 2.31–3.00 indicated that the algorithm may be adequate to classify the trait into high and low categories, however, each QTLs' significance and location should be further validated; 3.01–5.00 indicated that the algorithm provided sufficiently accurate data for reliable QTL analysis; >5.01 indicated that the algorithm was highly correlated with low error compared to the reference method, and was highly applicable for predicting traits for genomic studies.

QTL analysis

Spatial models were fitted for each trait/environment combination using GenStat (version 15.1.0.7720), where non-genetic components, such as spatial variation, were estimated. GenStat was used to calculate the heritability (h^2) and predicted means, Best Linear Unbiased Estimates (BLUEs) of each quality trait, and the correlation coefficients between environments. QTLs for the predicted means of grain yield, flowering date, grain texture, grain size and malting quality traits were resolved by performing Composite Interval Mapping (CIM). Windows QTL Cartographer (version 2.5_011) model 6 was used and optimised with 5–10 markers to control for the genetic background on a 10-cM window size (Hervé et al. 2001). The LOD scores for QTL detection were calculated based on 1,000 permutations at $P \leq 0.05$ for CIM, with a walk speed of 2 cM, and a threshold was considered significant when the LOD score was greater than 2.5 to minimise the risk of a Type II error (i.e. not detecting a true QTL). The percentage of the phenotypic variation explained by each QTL was estimated as the coefficient of determination (R^2). Consensus QTLs for a trait were considered to be significant and consistent if they were supported by at least two environments.

Previously published gene and QTL data related to plant development and malting quality were integrated into the V × B map based on their presence in the BOPA1 platform or their position was inferred based on the barley consensus map (Muñoz-Amatriaín et al. 2011) and OWB integrated map (Szűcs et al. 2009).

The V × B genetic linkage map and the indentified and aligned QTLs were drawn using MapChart (Voorrips 2002).

Results

Validation of the non-destructive phenotypic analyses

Phenotypic measurements were obtained using NIR and DIA and compared to those obtained using reference methods. Significant correlations were found for all malt

quality and grain size trait values, with the coefficients of determination (R^2) ranging from 0.82 (grain density) to 0.99 (grain major-width and GPC), with low SEPs (Fig. 1).

The RPD values for estimating grain length, volume and GPC were greater than 5.01 (Fig. 1a, c, f), and indicated that the algorithms were highly applicable for predicting traits for QTL analyses. The RPD values for major-width, minor-width, and malt extract were greater than 3.41 (Fig. 1a–d, f, g), and indicated that the algorithms were sufficiently accurate to use for genomic analyses. The grain density predictions, calculated using 3-D DIA grain volume and grain weight, were adequate for use in genomic studies with an acceptable error (RPD = 2.31, SEP = 0.011), when compared to gas displacement measurements (Fig. 1e).

Phenotypic characterisation

The parents of the DH population, Vlamingh and Buloke, were significantly different for endosperm hardness, grain density, malt extract, grain weight, volume, length and minor-width in all three environments, and for grain major-width in two environments (Table 1). Vlamingh and Buloke were not significantly different from each other for grain yield and flowering date (Table 1). Vlamingh had a softer and less dense endosperm with shorter, wider and, therefore, more spherical grain. Buloke had a higher malt extract content, grain weight, length and volume.

The DHLs grown in all three environments were more diverse in endosperm hardness, grain density, malt extract, GPC, grain weight, volume, length, minor, major-width, yield, and flowering date than the parents, indicating transgressive segregation (Table 1). All traits were normally distributed within the DHLs at each environment (supplemental Fig. 1).

Samples grown at Lake Bolac (rainfed) and Horsham (irrigated) had higher grain weight, larger grain volume, wider grain and softer endosperms than those grown at Horsham (rainfed) (Table 1). All three environments had similar distribution ranges of malt extract and GPC.

All phenotypes, with the exception of GPC, were significantly correlated ($P < 0.001$) between the environments (Table 2). Significant negative correlations ($P < 0.001$) were observed across all three environments for: grain density and minor-width; grain density and weight; density and major-width; density and volume; endosperm hardness and major-width; and, malt extract and GPC. Significant positive correlations ($P < 0.001$) were observed across all environments between the grain size traits: grain weight and grain volume; grain major and minor-width; major-width and volume; major-width and weight; length and volume; length and weight; and, volume and minor-width. Significant correlations ($P < 0.01$) were found among all three environments between: grain minor-width and volume; minor-width and weight; and, endosperm hardness and minor-width. Correlations were reported at two of the three environments between endosperm hardness and grain density, and, also, for endosperm hardness and malt extract.

QTL mapping

In total, 232 significant QTLs were detected for the 11 grain traits across the three environments assessed (supplemental Table 1). Consensus QTLs (i.e. QTLs identified at more than two environments) for grain texture, grain size, malting extract, GPC, grain yield and flowering date traits were identified on all seven chromosomes (Table 3).

Genes reported to influence plant development and malting quality that may influence grain texture, size, shape, yield and flowering date found were identified (Table 4). The genomic locations for these candidate genes were identified within the 'Vlamingh' × 'Buloke' (V × B) map using SNP markers to align their locations from barley consensus maps and other barley gene studies (Fig. 2).

Chromosome 1H

QTLs associated with endosperm hardness were identified on chromosome 1H, which accounted for 10.8–21.7 % of the phenotypic variance (Fig. 2; Table 3). The nearest marker to the QTL peaks was 2_1000 in all three environments, with the donor alleles contributed from the harder endosperm parent, Buloke, decreasing SKCS hardness by 2.08–2.72 units (Table 3; supplemental Table 1). QTLs were also detected for malt extract, flowering date and grain yield in this genomic region, but were only reported at one environment (irrigated Horsham, supplemental Table 1). The *Aglu3* and *HvBDG* genes (Table 4) were coincident with this genomic region (Table 4; Fig. 2). The consensus QTLs for endosperm hardness on 1H had the highest LOD scores compared with other genomic associations with endosperm hardness within the V × B DH mapping population.

In close proximity to this genomic region were consensus QTLs associated with grain volume and length (Fig. 2). Buloke with longer and larger grain than Vlamingh, contributed the alleles which decreased grain size at these loci. These QTLs were coincident with the *Vrs3* and *int-a* loci, which are reported to control vernalisation and alter the size of lateral spikelets (Table 4; Fig. 2).

Table 1 Mean values and standard errors for endosperm hardness, grain density, malt extract, grain protein content, grain weight, grain volume, grain length, major-width, minor-width, grain yield and flowering date in the 'Vlamingh' × 'Buloke' DH mapping population

	Endosperm hardness (SK units)	Grain density (g/mm^3)	Malt extract (% db)	Grain protein (% db)	Grain weight (mg)	Grain volume (mm^3)	Grain length (mm)	Major-width (mm)	Minor-width (mm)	Grain yield (t/ha)	Flowering date (JD)
Lake Bolac, rainfed											
Vlamingh	47.61[a]	1.384[a]	78.73[a]	14.09	46.00[a]	33.24[a]	7.84[a]	3.81[a]	2.65[a]	3.12	–
Buloke	51.77[a]	1.391[a]	79.81[a]	13.68	50.99[a]	37.42[a]	8.94[a]	3.78[a]	2.56[a]	2.86	–
DHL maximum	66.70	1.413	81.95	16.27	54.07	41.69	9.18	3.94	2.77	4.27	–
DHL minimum	35.83	1.367	76.24	12.31	39.04	28.23	7.70	3.56	2.39	1.89	–
DHL average	49.83	1.391	78.78	14.14	47.29	34.79	8.41	3.76	2.57	3.14	–
Least significant difference ($P < 0.01$)	2.21	0.004	0.74	0.63	2.10	0.46	0.11	0.02	0.04	1.61	–
Heritability (h^2)	0.78	0.72	0.15	0.12	0.87	0.93	0.88	0.90	0.83	0.31	–
Horsham, rainfed											
Vlamingh	57.87[a]	1.426[a]	79.60[a]	13.76	37.92[a]	27.27[a]	7.59[a]	3.63	2.29[a]	2.53	272.8
Buloke	62.58[a]	1.431[a]	80.80[a]	13.45	41.66[a]	30.85[a]	8.57[a]	3.62	2.25[a]	2.43	274.6
DHL maximum	78.16	1.453	82.12	16.45	46.15	32.85	8.87	3.76	2.53	3.37	278.6
DHL minimum	45.90	1.399	76.42	11.94	34.55	24.48	7.39	3.40	2.11	1.13	268.3
DHL average	61.99	1.428	79.32	14.09	39.44	28.82	8.12	3.59	2.26	2.30	273.3
Least significant difference ($P < 0.01$)	1.75	0.004	0.60	0.63	1.12	0.63	0.04	0.04	0.04	0.56	4.0
Heritability (h^2)	0.80	0.60	0.79	0.75	0.75	0.90	0.93	0.76	0.63	0.61	0.65
Horsham, irrigation											
Vlamingh	41.43[a]	1.396[a]	78.70[a]	13.82[a]	44.04[a]	32.23[a]	7.89[a]	3.75[a]	2.53[a]	4.17	275.0
Buloke	55.22[a]	1.405[a]	80.11[a]	12.95[a]	47.98[a]	36.21[a]	9.15[a]	3.70[a]	2.42[a]	3.54	275.8
DHL maximum	62.22	1.431	82.98	16.35	52.30	39.04	9.43	3.90	2.69	5.09	283.3
DHL minimum	31.96	1.377	76.00	11.41	38.09	27.80	7.68	3.48	2.19	2.16	269.0
DHL average	47.32	1.405	79.04	13.62	45.44	33.84	8.53	3.71	2.46	3.48	275.3
Least significant difference ($P < 0.01$)	2.10	0.007	0.60	0.53	1.82	1.02	0.14	0.04	0.04	1.68	1.8
Heritability (h^2)	0.77	0.66	0.68	0.61	0.68	0.67	0.81	0.50	0.57	0.27	0.75

[a] Vlamingh and Buloke were significantly different at the 0.01 probability level

Chromosome 2H

Consensus QTLs for grain density were located near the centromere of chromosome 2H, where Buloke contributed the alleles at these loci for decreasing grain density. There were two significant genomic regions between markers 1_1072–1_0651 and 2_1258–2_0960, explaining 4.0–8.8 % of the phenotypic variation (Table 3). Consensus QTLs were detected for grain volume, grain length, grain weight, and flowering date and were coincident with the grain density QTLs. Alleles from Vlamingh increased grain volume, grain weight and grain length, whereas alleles from Buloke decreased flowering date (Table 3; Fig. 2). The grain size QTLs accounted for a large portion of the phenotypic variation; for grain volume 16.6–19.5 %, for grain weight 6.6–12.5 %, and for grain length 17.1–24.7 % (Table 3). The consensus QTLs associated with flowering date accounted

7.2–8.7 % of the phenotypic variation. This genomic region was also in close proximity (∼7 cM) with the barley *cellulose synthase-like* (*HvCsIF*) genes (Table 4; Fig. 2).

Consensus QTLs for malt extract were reported on the arm 2HL at two environments, where Buloke contributed the alleles decreasing extract content, and was located between markers 2_1459–2_0715. In close proximity (∼15 cM) was the *Aglu5* gene.

Chromosome 3H

On the long arm of chromosome 3H, consensus QTLs were detected for grain length and grain volume. Two QTLs were found for grain length (marker intervals 2_0662–2_0612; and 2_1272–2_0339). One QTL was identified for grain volume (marker interval 2_0612–2_1272). Buloke contributed the alleles for the three consensus QTLs reported,

Table 2 Correlations coefficients (*r*) for endosperm hardness, grain density, malt extract, grain protein content, grain weight, grain volume, grain length, major-width, and minor-width in the 'Vlamingh' × 'Buloke' DH mapping population grown at three environments

		Density			Extract			Weight			Hard			Length			Major			Minor			Protein			Volume	
		HO	IH	LB	HO	IH	LB	HO	IH	LB	HO	IH	LB	HO	IH	LB	HO	IH	LB	HO	IH	LB	HO	IH	LB	HO	IH
Density	IH	0.41**																									
	LB	0.44**	0.56**																								
Extract	HO	0.07	0.05	0.07																							
	IH	−0.13	−0.01	−0.06	0.55**																						
	LB	−0.02	0.20*	0.25**	0.22**	0.25**																					
Weight	HO	−0.50**	−0.21**	−0.27**	0.03	−0.06	−0.06																				
	IH	−0.30**	−0.65**	−0.42**	−0.07	0.02	−0.12	0.67**																			
	LB	−0.23**	−0.30**	−0.59**	−0.10	−0.09	−0.20*	0.68**	0.68**																		
Hard	HO	0.22**	0.27**	0.21*	−0.29**	−0.05	−0.09	−0.07	0.00	0.08																	
	IH	0.11	0.34**	0.25**	−0.22**	−0.12	−0.09	−0.08	0.07	0.07	0.66**																
	LB	0.09	0.27**	0.30**	−0.09	−0.26**	−0.20*	−0.13	−0.08	−0.08	0.59**	0.63**															
Length	HO	0.24**	0.09	0.10	−0.18*	−0.12	−0.09	0.50**	0.57**	0.57**	0.37**	0.31**	0.18*														
	IH	0.16	0.10	0.09	0.10	0.09	0.03	0.55**	0.56**	0.56**	0.35**	0.35**	0.17**	0.89**													
	LB	0.18*	0.10	0.10	0.09	0.03	0.02	0.46**	0.59**	0.59**	0.39**	0.33**	0.18*	0.87**	0.86**												
Major	HO	−0.48**	−0.23**	−0.37**	0.08	0.09	0.06	0.34**	0.37**	0.37**	−0.42**	−0.24**	−0.26**	−0.09	−0.23**	0.24**											
	IH	−0.43**	−0.61**	−0.51**	0.09	0.03	0.02	0.52**	0.30**	0.30**	−0.45**	−0.35**	−0.39**	−0.23**	0.23**	0.17**	0.70**										
	LB	−0.36**	−0.41**	−0.68**	0.03	0.03	0.02	0.45**	0.45**	0.65**	−0.31**	−0.39**	−0.30**	−0.04	0.05	0.04	0.71**	0.67**									
Minor	HO	−0.45**	−0.52**	−0.52**	0.11	0.03	0.03	0.38**	0.38**	0.54**	−0.31**	−0.15	−0.18*	−0.14	0.12	0.12	0.56**	0.56**	0.55**								
	IH	−0.45**	−0.90**	−0.60**	0.00	0.09	0.09	0.57**	0.27**	0.18*	−0.43**	−0.43**	−0.20*	−0.20*	0.23**	0.23**	0.76**	0.76**	0.49**	0.55**							
	LB	−0.41**	−0.57**	−0.91**	−0.08	0.00	−0.06	0.40**	0.59**	0.59**	−0.25**	−0.27**	−0.37**	−0.12	0.13	0.09	0.43**	0.56**	0.79**	0.56**	0.65**						
Protein	HO	0.13	−0.17*	−0.11	−0.64**	−0.23**	−0.15	−0.09	0.11	0.08	−0.05	−0.05	0.04	0.05	0.02	0.01	0.06	0.06	0.05	0.15	0.02	0.21*					
	IH	0.07	0.06	−0.01	−0.28**	−0.10	−0.35**	−0.01	0.01	−0.02	−0.01	−0.01	0.00	0.02	0.01	0.02	0.05	0.05	0.01	0.01	0.16	0.02	0.28**				
	LB	−0.01	−0.15	0.04	−0.14	−0.04	−0.10	0.16	−0.02	0.08	−0.07	−0.01	0.07	0.07	0.07	0.08	0.04	0.05	0.15	0.04	0.16	0.08	0.14	0.03			
Volume	HO	−0.42**	−0.55**	−0.33**	0.02	−0.03	−0.04	0.89**	0.70**	0.73**	−0.04	−0.07	−0.05	0.52**	0.58**	0.54**	0.65**	0.37**	0.51**	0.58**	0.20*	0.31**	0.12	0.10	0.31**	0.72**	
	IH	−0.24**	−0.38**	−0.38**	−0.10	−0.04	−0.16	0.68**	0.88**	0.68**	−0.12	−0.12	−0.13	0.58**	0.52**	0.58**	0.52**	0.52**	0.47**	0.36**	0.50**	0.37**	0.01	0.01	0.50**	0.76**	0.73**
	LB	−0.27**	−0.35**	−0.61**	−0.12	−0.06	−0.10	0.70**	0.94**	0.70**	0.01	−0.02	−0.18*	0.55**	0.54**	0.50**	0.43**	0.36**	0.70**	0.41**	0.31**	0.62**	0.03	0.06	0.41**	0.72**	0.73**

Correlations tested using a two-tailed test at the significance levels 0.001 (double asterisks) and 0.01 (asterisks)

HO Horsham rainfed, *IH* Horsham irrigated, *LB* Lake Bolac rainfed, *Density* grain density (g/mm³), *Extract* malt extract content (%, db), *Weight* grain weight (mg), *Hard* endosperm hardness (SKCS units), *Length* grain length (mm), *Major* grain major-width (mm), *Minor* grain minor-width (mm), *Protein* grain protein content (%, db), *Volume* grain volume (mm³)

Table 3 Consensus genomic regions of the significant QTLs for endosperm hardness, grain density, grain volume, malt extract, grain protein, grain weight, grain length, major-width, and minor-width analysed in the 'Vlamingh' × 'Buloke' DH mapping population across at least two environments

Chr	Env	Position (cM)	LOD	% Var exp, R^2	Additive effect	Higher parent	Marker interval
Endosperm hardness (SK units)							
1	3	61.2–65.2	8.8–19.9	10.8–21.7	−(2.72–2.08)	Buloke	2_1000–1_0552
4	2	133.9	2.7–4.1	3.2–4.8	1.00–1.33	Vlamingh	2_0044–2_0007
5	3	33.2–43.8	4.3–9.4	4.3–12.5	1.24–2.06	Vlamingh	2_0386–2_0306
7	2	40.3–42.3	5.1–7.3	5.3–6.8	1.39–1.46	Vlamingh	2_0993–1_0056
Grain density (g/mm³)							
2	2	77.3–79.3	3.1–7.3	4.0–8.8	−(0.014–0.008)	Buloke	1_1072–1_0651
2	2	85.7–86.7	4.6–5.6	5.7–6.8	−(0.012–0.010)	Buloke	2_1258–2_0960
6	2	115.9–123.9	3.1–6.3	3.9–8.2	−(0.012–0.007)	Buloke	2_1224–2_0687
Malt extract (% w/w, dry basis)							
2	2	168.1–170.1	5.1–5.2	4.7–5.3	−(0.25–0.24)	Buloke	2_1459–2_0715
4	2	44.7–54.1	4.2–13.1	7.9–13	0.33–0.42	Vlamingh	2_1122–1_0411
4	2	62.4–63.8	4.4–10.1	3.5–10.3	0.23–0.39	Vlamingh	1_1244–2_0361
4	2	93.6–95.6	2.7–3.7	2.6–3.5	0.18–0.23	Vlamingh	2_1332–1_0588
Protein (% w/w, dry basis)							
4	2	98.2–105.9	3.3–6.5	4.0–6.8	−(0.21–0.16)	Buloke	2_1332–2_0732
Grain weight (mg)							
2	3	70.1–72.5	6.3–12.6	6.6–12.5	0.62–0.80	Vlamingh	2_0039–1_1072
4	3	51.3–55.5	3.4–8.7	3.1–9.7	−(0.87–0.37)	Buloke	2_1122–1_0946
5	3	58.6–60.5	5.3–9.3	5.8–9.1	0.57–0.76	Vlamingh	2_1239–2_0367
5	2	91.8	4.6–4.7	5.3–6.7	−(0.78–0.53)	Buloke	1_1290–1_1350
5	3	105.8	3.3–6.7	3.3–7.3	−(0.85–0.46)	Buloke	2_0320–1_0477
5	3	127.2–132.9	3.8–5.9	3.6–6.4	0.44–0.97	Vlamingh	1_1456–1_0095
5	2	140.5–145.3	5.5–7.1	6.7–8.3	0.72–0.76	Vlamingh	1_0095–1_1071
6	2	113.9	2.6–5.9	2.6–5.6	0.39–0.49	Vlamingh	2_1224–2_0558
Grain volume (mm³)							
1	2	47.4	3.0–3.3	2.4–2.7	−(0.31–0.25)	Buloke	2_1048–2_0514
2	3	70.1–79.3	16.6–19.5	15.3–19.3	0.71–0.90	Vlamingh	2_0039–1_0265
3	2	156.5–158.5	3.7–4.2	3.3–3.4	−(0.37–0.37)	Buloke	2_0612–2_1272
4	3	0.0–1.7	2.6–5.5	2.1–4.6	0.29–0.32	Vlamingh	2_1056–1_1345
5	2	87.8–89.8	2.7–6.6	2.8–7.3	−(0.48–0.38)	Buloke	2_1480–1_1350
5	3	102.0–105.8	4.1–8.3	3.4–7.2	−(0.50–0.44)	Buloke	1_0834–1_0477
5	2	159.9–160.8	3.5–3.6	2.9–2.9	0.36–0.39	Vlamingh	2_0100–1_1497
6	3	121.2–135.7	4.8–11.6	4.4–10.2	0.44–0.64	Vlamingh	2_0558–2_0687
Grain length (mm)							
1	3	34.7–47.4	5.7–7.3	3.4–5.4	−(0.07–0.07)	Buloke	1_0186–2_0514
2	3	70.1–72.5	24.6–33.2	19.2–24.7	0.15–0.18	Vlamingh	2_0039–1_1072
2	3	79.3–80.5	21.3–29.5	17.1–23.3	0.14–0.18	Vlamingh	1_1072–2_1258
3	2	133.0–137.0	2.7–6.3	1.6–4.8	−(0.08–0.04)	Buloke	2_0662–2_0612
3	3	172.3–178.6	3.9–4.6	2.5–2.7	−(0.06–0.05)	Buloke	2_1272–2_0339
5	2	45.8–47.8	26.5–28.1	22.4–22.6	0.14–0.17	Vlamingh	2_0306–2_0306
5	2	54.6	10.1–17.3	7.2–13.8	0.10–0.13	Vlamingh	1_0641–2_1239
5	2	172.3–172.6	2.6–3.0	1.5–1.9	−(0.05–0.04)	Buloke	1_1490–2_0934
6	2	104.6–106.6	8.9–11.9	9.3–9.9	0.10–0.11	Vlamingh	2_1224–2_0355
6	3	120.3–123.9	6.6–10.5	3.9–7.1	0.08–0.10	Vlamingh	2_0558–2_0005
7	3	141.3–161.2	3.3–6.6	2.0–4.4	0.05–0.07	Vlamingh	2_0808–1_1243

Table 3 continued

Chr	Env	Position (cM)	LOD	% Var exp, R^2	Additive effect	Higher parent	Marker interval
Grain major-width (mm)							
4	2	18.3	5.7–7.9	5.4–7.9	0.02–0.02	Vlamingh	1_0113–2_0557
5	3	34.6–39.8	5.5–6.6	5.4–6.3	−(0.02–0.02)	Buloke	1_0157–2_0306
5	2	91.8	3.8–9.0	4.5–11.8	−(0.03–0.02)	Buloke	1_1290–1_1350
5	3	104–109.8	5.8–14.2	5.5–18.2	−(0.03–0.02)	Buloke	1_0024–1_0477
5	3	132.9–147.3	6.8–13.7	6.8–14.6	0.02–0.03	Vlamingh	1_1456–1_1071
5	2	153.9–155.9	7.4–13.9	9.9–15.5	0.02–0.03	Vlamingh	1_1071–2_0100
Grain minor-width (mm)							
4	2	53.0–55.5	2.7–10.3	2.6–10.8	−(0.03–0.01)	Buloke	2_1122–1_0946
5	3	43.8	3.9–11.2	5.7–12.9	−(0.03–0.01)	Buloke	1_1506–2_0306
5	2	143.3–145.3	3.3–9.2	3.2–10.6	0.02–0.03	Vlamingh	1_1375–1_1071
5	2	157.9–160.8	6.1–10.4	7.8–13.1	0.02–0.03	Vlamingh	2_0100–1_1490
Grain yield (t/ha)							
5	2	29.4–29.4	3.3–4.0	6.3–6.7	0.13–0.09	Buloke	2_0386–2_0332
7	2	31.2–40.3	3.2–6.8	6.1–10.1	0.12–0.17	Vlamingh	2_0014–1_0056
Flowering date (JD)							
2	2	70.4–72.5	8.2–8.8	7.2–8.7	0.71–0.59	Buloke	2_0160–2_0690
4	2	122.0–129.9	3.0–3.0	3.1–7.6	0.78–0.34	Buloke	1_0510–2_0044
7	2	39.5–40.3	17.7–32.0	20.7–31.6	0.87–1.57	Vlamingh	2_0993–2_1528

Chr chromosome number, *Env* number of environments, *Position* position of the QTL on the chromosome (cM), *LOD* logarithm of the odds, *% Var exp, R^2* the percentage of phenotypic variation explained attributed to that QTL, *Additive effect* the mean difference between the two parental allele classes, measured in the units of the trait, *Higher parent* contributing the alleles, and *Marker interval* size of the QTL

decreasing the grain length and volume. One locus associated with grain length (marker interval 2_0662–2_0612) was also coincident with QTLs found in one environment for endosperm hardness, malt extract, grain yield and flowering date (supplementary Table 1).

Chromosome 4H

On the long arm of chromosome 4H, consensus QTLs were found for endosperm hardness, with the nearest marker being 2_0044 (Fig. 2; supplemental Table 1). These QTLs explained between 3.2 and 4.8 % of the phenotypic variance, and the Vlamingh alleles contributed to an increase in hardness of 1.00–1.33 SKCS units (Table 3). This genomic region was coincident with consensus QTLs associated with flowering date, and the vernalisation gene, *VRN-H2*, and the *Bmy1* gene (Table 4; Fig. 2). QTLs associated with grain major-width and grain yield, in one environment, were also coincident with this region (supplemental Table 1).

Three genomic regions were reported to have consensus QTLs for malt extract on chromosome 4H at all three environments. Vlamingh, lower in malt extract, contributed the alleles that increased extract for all QTLs observed on this chromosome (Table 3). The first genomic region for malt extract, with the marker interval 2_1122–1_0411, explained between 7.9 and 13.0 % of the phenotypic

variation. Consistent QTLs, reported for grain weight and grain minor-width, were detected in this first genomic region associated with malt extract (Fig. 2). The Buloke allele decreased the grain minor-width and weight among these QTLs associated with grain size. This first genomic region, associated with malt extract and grain size, was coincident with the *PhyA* and *PhyB* genes, which are involved in the regulation of flowering, and the *QTL2* gene associated with malting quality (Table 4; Fig. 2). The second genomic region on chromosome 4H, near the centromere between markers 1_1244 and 2_0361, reported at two environments, was associated with malt extract (Fig. 2). This was coincident with the *DTDP* gene (Table 4; Fig. 2). The third genomic region on the long arm of chromosome 4H, significant for malt extract, was coincident with significant QTLs for GPC (Fig. 2).

On the short arm of chromosome 4H, there were two genomic regions associated with grain volume and grain major-width. Vlamingh contributed the alleles in both regions, increasing grain size.

Chromosome 5H

On chromosome 5H, there were many consensus QTLs associated with endosperm hardness, grain major-width, minor-width, weight, length, and volume.

Table 4 Published genes and QTL data in barley associated with plant development and malting quality, and their reported functionality

Chromosome	Gene/loci	Functionality	References
1H	*Vrs3*	Morphology modifying gene in two-row/six-row segregating crosses	Chutimanitsakun et al. (2011)
1H	*int-a*	Intermedium spike-a, alter the size of lateral spikelets	Komatsuda and Mano (2002)
1H	*Aglu3*	α-Glucosidase	Szűcs et al. (2009)
1H	*HvBDG*	(1-3,1-4)-β-D-Glucanase	March et al. (2012) and Walker et al. (2011)
2H	*PPD-H1*	Photoperiod response	Karsai et al. (2008)
2H	*HvCslF*	Barley cellulose synthase-like genes: HvCslF3, HvCslF4, HvCslF8, and HvCslF10	Burton et al. (2006)
2H	*Vrs1*	The presence of a recessive allele at the *Vrs1* locus is sufficient to cause the wild type two rowed barley to become six-rowed barley	Cuesta-Marcos et al. (2010) and Komatsuda et al. (1999)
2H	*Aglu5*	α-Glucosidase	Szűcs et al. (2009)
4H	*int-c*	Intermedium spike-c, alter the size of lateral spikelets	Ramsay et al. (2011)
4H	QTL2	Moderate effects on several malting quality traits; malt extract, diastatic enzyme activity, malt β-glucan content, and seed dormancy	Gao et al. (2004)
4H	*PhyA*	Phytochromes, involved in regulation of flowering time	Szűcs et al. (2006)
4H	*PhyB*	Phytochromes, involved in regulation of flowering time	Szűcs et al. (2006)
4H	*DTDP*	dTDP-glucose dehydratase	Szűcs et al. (2009)
4H	*VRN-H2*	Vernalisation response (formerly *Sgh1*)	Szűcs et al. (2007)
4H	*Bmy1*	β-Amylase	Szűcs et al. (2009)
5H	*Ha* locus	Reported to modify grain hardness (hordoindolines)	Beecher et al. (2002) and Caldwell et al. (2004)
5H	*Fr-2/CBR_cluster*	Frost resistance and dehydration responsive elements (DRE)-binding factors	Galiba et al. (2009)
5H	*VRN-H1*	Vernalisation response, determinant in flowering time (formerly *Sgh2*)	Karsai et al. (2008) and Szűcs et al. (2007)
5H	*PhyC*	Phytochromes, involved in regulation of flowering time	Szűcs et al. (2006)
7H	*VRN-H3*	Vernalisation response, determinant in flowering time	Karsai et al. (2008)
7H	*CAT1*	Catalase 1	Szűcs et al. (2009)

QTLs for endosperm hardness were detected near the centromere of chromosome 5H at all three environments, these consensus QTLs explained 4.3–12.5 % of the phenotypic variation. These QTLs showed an increase in endosperm hardness contributed from the Vlamingh alleles (Table 3). In this region, coincident QTLs were significantly associated with grain minor-width, major-width, length and grain yield (Fig. 2). Grain length explained between 22.4 and 22.6 % of the phenotypic variation, major-width explained between 5.4 and 6.3 %, and minor-width between 5.7 and 12.9 %. The alleles for increased grain length were from Vlamingh, the shorter grained parent; and, the alleles which decreased grain minor and major-width were from Buloke, the parent with the thinner grain. The location of the *Ha* locus, according to the barley consensus map, when aligned to the V × B map, was not coincident (∼38.5 cM apart) with the consensus QTL associated with endosperm hardness on the short arm of 5H near the centromere (Fig. 2).

Consensus QTLs detected on chromosome 5H, between markers 1_0641 and 2_0367, were associated with grain length and grain weight. These explained between 7.2–13.8 % (grain length) and 5.8–9.1 % (grain weight) of the phenotypic variation, and the alleles contributed from Vlamingh increased grain length and weight.

The most significant consensus QTLs associated with grain minor-width and major-width, were reported on the long arm of chromosome 5H, located in the marker interval between 1_1071 and 1_1490. The QTLs accounted for 7.8–13.1 % of the phenotypic variation for grain minor-width, and 9.9–15.5 % for the major-width. A consensus QTL associated with grain volume was also reported in this region, and in close proximity (∼20 cM) to this region were QTLs associated with grain length. Alleles from Vlamingh increased grain minor-width, major-width, and volume, and alleles from Buloke decreased grain length (Table 3).

Within the marker interval 1_1456–1_1071, there were consensus QTLs that also had a positive effect on grain minor and major-width, and grain weight. These QTLs explained between 6.8 and 14.6 % for major-width, 3.2 and 10.6 % for minor-width and 3.6 and 8.3 % for grain weight. Vlamingh contributed all the alleles for these traits. The QTLs were coincident with a flowering date QTL (supplementary Table 1), and the vernalisation gene (*VRN-*

Fig. 2 The genetic map of the 'Vlamingh' × 'Buloke' DH mapping population and associated consensus quantitative trait loci (QTLs) observed for endosperm hardness (*hard*), grain density (*density*), grain volume (*volume*), malt extract (*extract*), grain length (*length*), minor-width (*minor*), major-width (*major*), and grain protein content (*protein*), across at least two environments (*blue*). QTLs observed for grain yield and flowering date, across at least two environments (*red*). Markers are presented to a scale of genetic distance (centiMorgans). Other loci related to malting quality and plant development were aligned based on the historical markers integrated into the barley consensus map (Muñoz-Amatriaín et al. 2011) or the OWB integrated map (Szűcs et al. 2009), and are presented on the *left* indicated by an *arrow in bold italics* (*green*) (colour figure online)
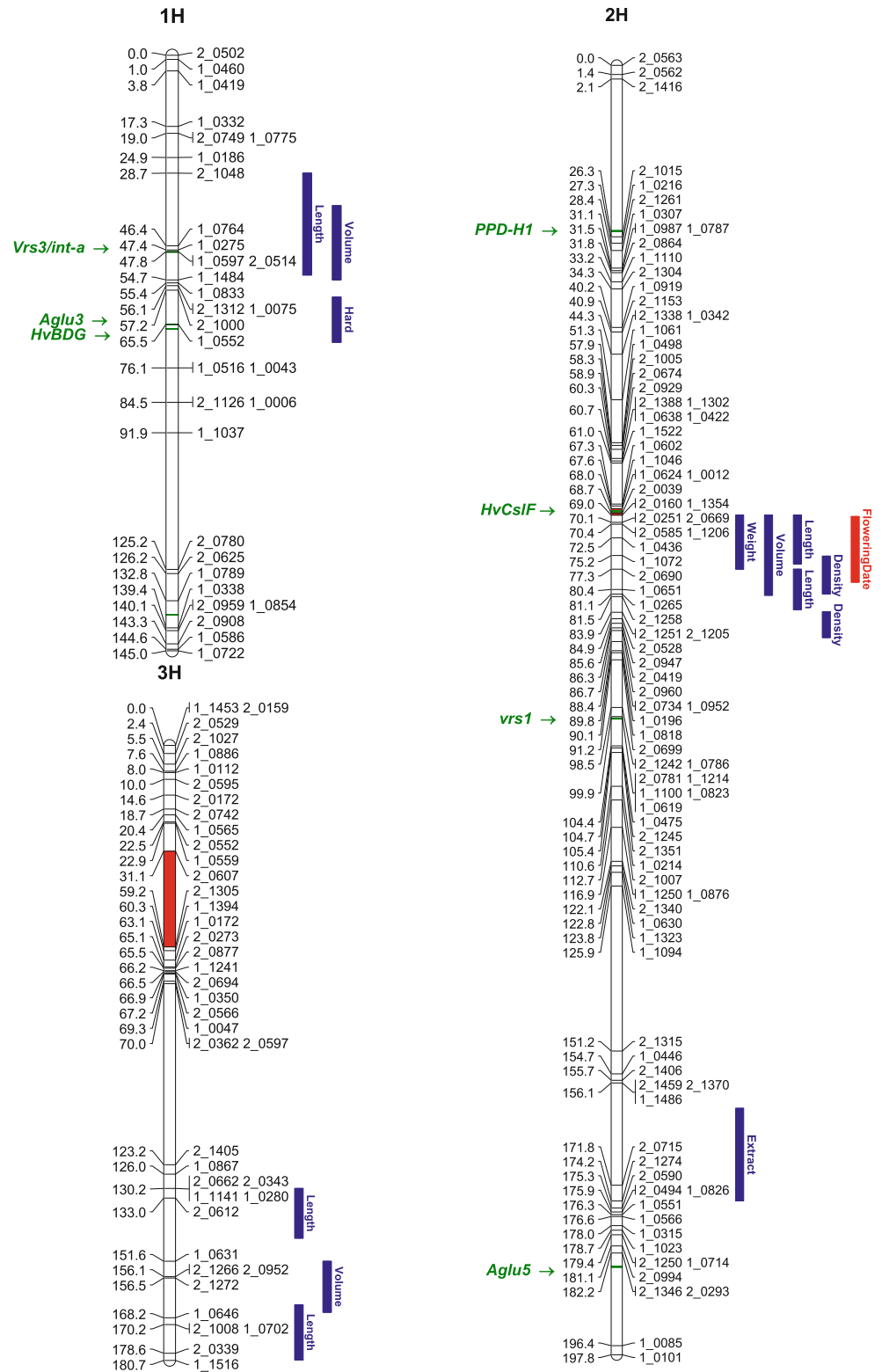
H1) and the *PhyC* gene, involved in the regulation of flowering (Table 4; Fig. 2).

Two genomic regions for grain major-width, grain volume and grain weight were detected within the marker interval 2_1480–1_4077. The contributing alleles were from Buloke in all traits, which decreased grain major-width, volume and weight. This region was coincident with the *Fr2* and *CBF_cluster* genes, which control frost resistance and dehydration responsive elements (DRE)-binding factors (Table 4).
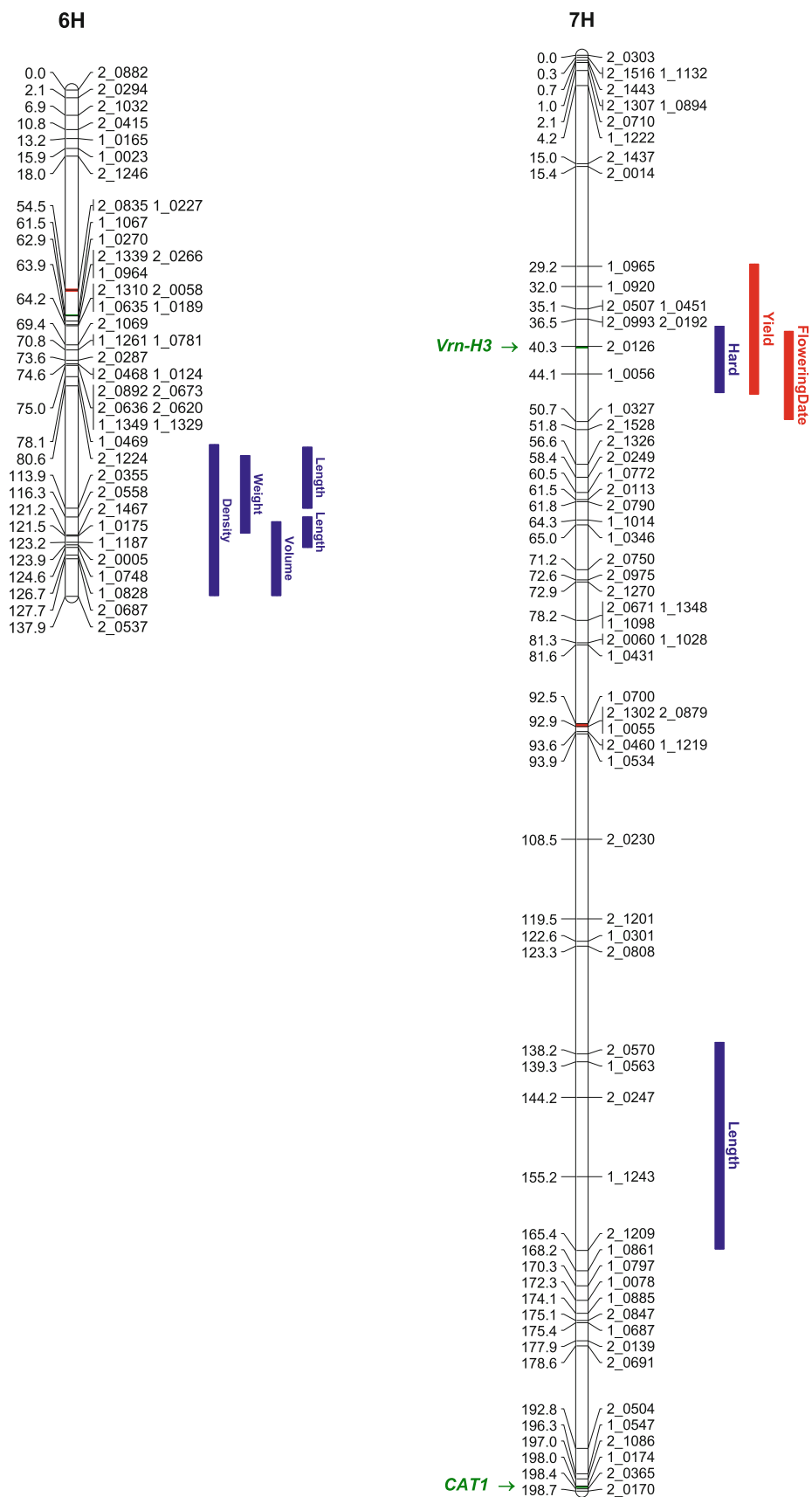
Fig. 2 continued
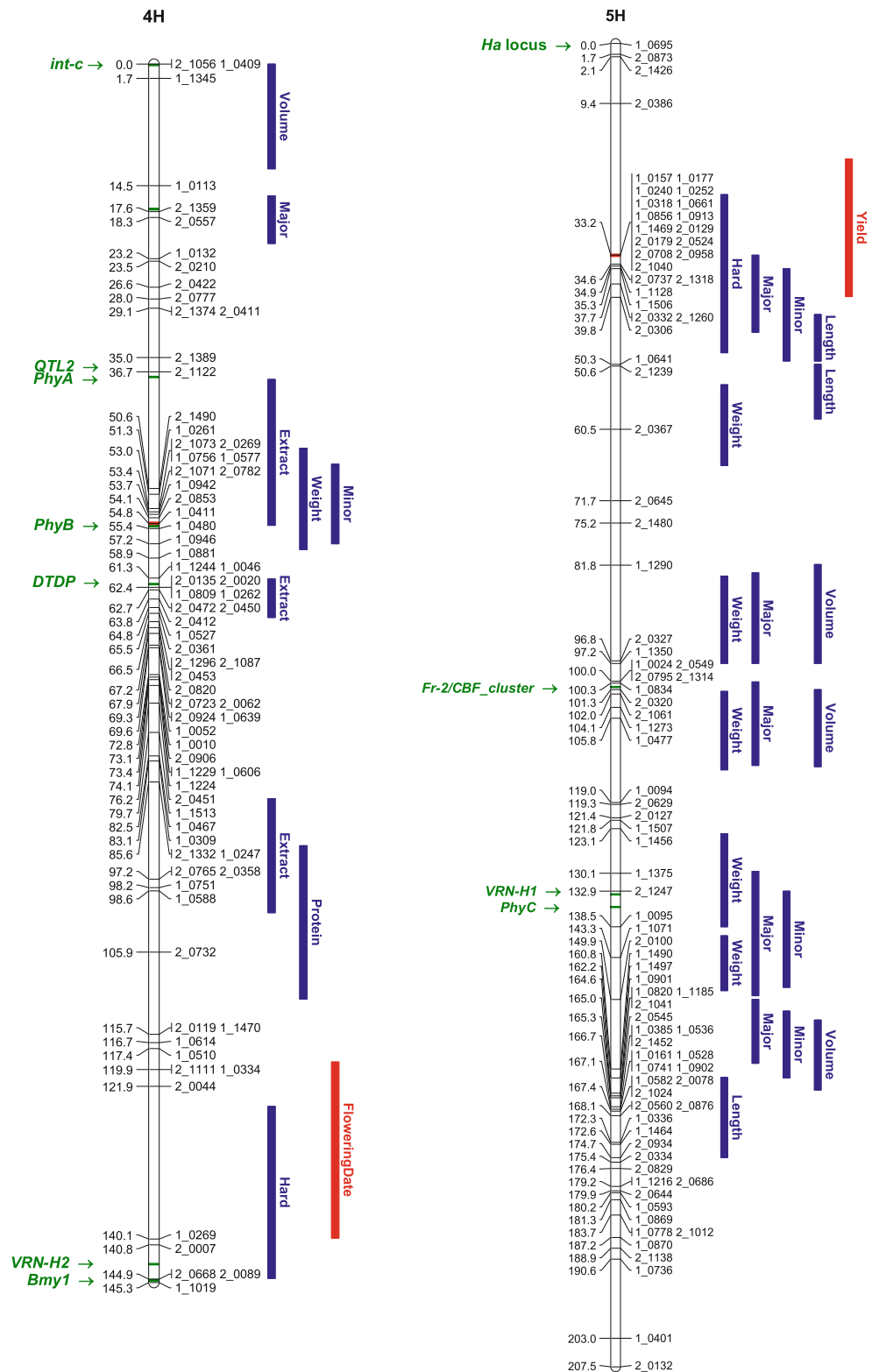
Chromosome 6H

A consensus genomic region that had significant QTLs for grain density was on the long arm on chromosome 6H. These QTLs explained 3.9–8.2 % of the phenotypic variation, where the Buloke alleles decreased grain density. The grain density QTLs on chromosome 6H were coincident with consensus QTLs detected for grain weight, length, and volume, where Vlamingh contributed the alleles increasing these grain weight and size traits (Table 3).

Fig. 2 continued

6H

Position	Marker
0.0	2_0882
2.1	2_0294
6.9	2_1032
10.8	2_0415
13.2	1_0165
15.9	1_0023
18.0	2_1246
54.5	2_0835 1_0227
61.5	1_1067
62.9	1_0270
63.9	2_1339 2_0266
	1_0964
64.2	2_1310 2_0058
	1_0635 1_0189
69.4	2_1069
70.8	1_1261 1_0781
73.6	2_0287
74.6	2_0468 1_0124
	2_0892 2_0673
75.0	2_0636 2_0620
	1_1349 1_1329
78.1	1_0469
80.6	2_1224
113.9	2_0355
116.3	2_0558
121.2	2_1467
121.5	1_0175
123.2	1_1187
123.9	2_0005
124.6	1_0748
126.7	1_0828
127.7	2_0687
137.9	2_0537

Density, Weight, Volume, Length, Length

7H

Position	Marker
0.0	2_0303
0.3	2_1516 1_1132
0.7	2_1443
1.0	2_1307 1_0894
2.1	2_0710
4.2	1_1222
15.0	2_1437
15.4	2_0014
29.2	1_0965
32.0	1_0920
35.1	2_0507 1_0451
36.5	2_0993 2_0192
Vrn-H3 → 40.3	2_0126
44.1	1_0056
50.7	1_0327
51.8	2_1528
56.6	2_1326
58.4	2_0249
60.5	1_0772
61.5	2_0113
61.8	2_0790
64.3	1_1014
65.0	1_0346
71.2	2_0750
72.6	2_0975
72.9	2_1270
78.2	2_0671 1_1348
	1_1098
81.3	2_0060 1_1028
81.6	1_0431
92.5	1_0700
	2_1302 2_0879
92.9	1_0055
93.6	2_0460 1_1219
93.9	1_0534
108.5	2_0230
119.5	2_1201
122.6	1_0301
123.3	2_0808
138.2	2_0570
139.3	1_0563
144.2	2_0247
155.2	1_1243
165.4	2_1209
168.2	1_0861
170.3	1_0797
172.3	1_0078
174.1	1_0885
175.1	2_0847
175.4	1_0687
177.9	2_0139
178.6	2_0691
192.8	2_0504
196.3	1_0547
197.0	2_1086
198.0	1_0174
198.4	2_0365
CAT1 → 198.7	2_0170

Yield, FloweringDate, Hard, Length

Chromosome 7H

Consensus QTLs associated with endosperm hardness were detected on the short arm of chromosome 7H for two environments, this explained 5.3–6.8 % of the phenotypic variance (Table 3). The alleles were contributed from the softer parent, Vlamingh, increasing endosperm hardness indicating transgressive segregation, and were located in the marker interval 2_0993–1_0056. These consensus hardness QTLs were coincident with consensus QTLs for grain yield and flowering date (Table 4; Fig. 2). The VRN-H3 gene was also coincident with this genomic region (Table 4; Fig. 2). Significant QTLs detected for grain length, major-width, minor-width and protein content from one environment were also coincident with this region associated with endosperm hardness (supplemental Table 1).

On the long arm of chromosome 7H, there were consensus QTLs detected for grain length (Fig. 2). These explained between 2.0 and 4.4 % of the phenotypic variation, where Vlamingh contributed the alleles which increased grain length. The QTLs were detected between the marker interval 2_0808–1_1243.

Discussion

The development of accurate high-throughput and non-destructive phenotyping tools, such as DIA (Williams et al. 2012) and NIR (Black and Panozzo 2002), will enhance the knowledge gained from genomics studies (Xu and Crouch 2008). Validating secondary techniques is essential in genomic studies to ensure significant and accurate regions are detected with confidence. In this study, validation statistics indicated that data from DIA and NIR were significantly correlated ($R^2 > 0.82$), with acceptable standard errors, compared to reference data (Fig. 1). For grain length, volume, and protein content, the algorithms were the most accurate, and highly applicable for use in genomic studies. Algorithms calculating grain major-width, minor-width, and malt extract were accurate for resolving significant QTLs (Table 3; Fig. 2). The algorithm used to calculate grain density was adequate for reporting significant genomic regions (Table 3; Fig. 2), as these regions were also coincident with grain weight and volume QTLs, located on chromosomes 2H and 6H.

The grain texture traits, endosperm hardness and grain density, were significantly inter-correlated in all three environments (Table 2). In a previous investigation, different grain components were found to interact independently of these traits, even though they were significantly correlated (Walker and Panozzo 2011). In this study, grain volume and weight were significantly correlated to grain density, but not endosperm hardness, and grain length was significantly correlated with endosperm hardness, but not grain density (Table 2). No coincident consensus QTLs were detected that control both grain density and endosperm hardness. For endosperm hardness, consensus QTLs were detected on chromosomes 1H, 4H, 5H and 7H, whereas for grain density, consensus QTLs were detected on chromosomes 2H and 6H. Therefore, even though endosperm hardness and grain density are interdependent traits (Walker and Panozzo 2011), different genomic regions can be manipulated independently to influence endosperm hardness or grain density.

There is an apparent association between the grain density and barley processing quality (Fang and Campbell 2000; Walker and Panozzo 2011). This association is supported by significant correlations between grain density and grain size traits (Table 2). Also, different grain components and genomic regions were found to be associated with grain density, compared with those associated with endosperm hardness. Therefore, understanding the genomic regions associated with grain density would add to our knowledge in understanding the impact of grain texture on processing quality. Grain density is calculated by dividing the weight by the volume. QTLs for grain weight and volume generally had higher LOD scores and smaller intervals, than those detected for grain density. Also, we detected a genomic region on the long arm of chromosome 5H, between markers 1_1290 and 1_0477, significantly associated with grain weight and volume but not grain density. This region, on 5H, is likely to be associated with grain density, but the estimate of grain density was not sensitive enough to detect significant QTL in this region. Coincident QTLs identified for both grain weight and volume may be sufficient to identify genomic regions associated with grain density, although this needs validation with other populations and environments.

The largest and most significant consensus QTLs associated with endosperm hardness for the V × B mapping population were found on chromosome 1H, where the alleles from Buloke decreased hardness. QTLs for endosperm hardness in the same region were also reported by Beecher et al. (2002) in the 'Steptoe' × 'Morex', Fox et al. (2007) in the 'Patty' × 'Tallon', and Walker et al. (2011) in the 'Arapiles' × 'Franklin' barley mapping populations. The traits, endosperm hardness and malt extract, were significantly correlated within all three environments, confirming a relationship between endosperm hardness and malt extract (Table 2). In this study, there was a significant QTL for malt extract in the same region of 1H, where favourable alleles increased extract (from Vlamingh). Malting quality QTLs were reported to be coincident with endosperm hardness QTLs in the 'Arapiles' × 'Franklin' (Walker et al. 2011; Panozzo et al. 2007) and

'Steptoe' × 'Morex' (Beecher et al. 2002) barley populations. Panozzo et al. (2007) suggested that there is benefit in using marker assisted selection (MAS) to manipulate malting quality in this genomic region on chromosome 1H. However, this region was also coincident with a flowering date QTLs for this DH population. Although this region is linked with endosperm hardness and malt extract, it may be an inappropriate region for MAS as the genes influencing grain quality may also be tightly linked to flowering date.

Consensus QTLs associated with endosperm hardness, grain weight, minor-width, major-width, and grain yield were coincident on the short arm of chromosome 5H. Grain weight has been shown to influence endosperm hardness in other studies (Turuspekov et al. 2008; Galassi et al. 2011; Chen et al. 2009). In addition, the short arm of chromosome 5H has been associated with *Hin* genes (Beecher et al. 2002; Chen et al. 2009; Galassi et al. 2011; Takahashi et al. 2010), which is considered by some researchers to control endosperm hardness (Beecher et al. 2002; Turuspekov et al. 2008). The location of the *Ha* locus in this study was not closely linked with the endosperm hardness QTLs (~38.5 cM apart; Fig. 2). Therefore, this study highlights the complexity of the relationships among endosperm hardness, grain yield, and grain size traits, and if endosperm hardness is in fact strongly linked to the functionality of the *Hin* genes.

Malt extract is a complex trait, where many genomic regions along the chromosome have been shown to influence various components associated with this trait. Consensus QTLs for malt extract content were not found to be coincident with consensus QTLs for endosperm hardness or grain density. In a recent study of structural components of the grain, endosperm hardness and grain density were found to influence water diffusivity but not influence the hydrolytic activities of enzymes involved in improving malt extract (Mayolle et al. 2012). In this study, QTLs for malt extract were coincident with regions associated with hydrolytic enzymes, namely chromosome 4HL (also coincident with GPC), 2H, and 1H (Panozzo et al. 2007; Szűcs et al. 2009; Collins et al. 2003). Also, malt extract QTLs on chromosome 4HS, near the centromere, were found to be coincident with QTLs associated with grain weight, minor-width and flowering date. Therefore, genomic regions associated with malt extract that are coincident with hydrolytic enzymes or grain size traits need to be further examined so that we can gain an understanding into the genetic basis of malting quality and how each region affects extract.

Studies in wheat using DIA have indicated that grain length and major-width are often not genetically associated (Campbell et al. 1999). Williams et al. (2012) detected independent QTLs for grain length, major-width and minor-width. In this study, QTLs were observed for different grain size components in different genomic regions using DIA. QTLs associated with grain length were detected on 7H, QTLs associated with grain length and volume were detected on chromosome 1H, QTLs associated with grain length, volume and weight were detected on chromosome 2H and 6H. QTLs associated with grain volume, major-width, minor-width and weight were detected on chromosome 4H, whereas QTLs associated with grain minor-width, major-width, weight and length were detected on 5H. However, many of these regions were associated with grain yield, flowering date and genes reported to influence plant development (Fig. 2). Often genomic regions associated with improved grain plumpness are also associated with grain yield, flowering date and/or malt-quality related traits. If genomic regions could be identified for grain length, major-width, minor-width or weight traits and were proven to be unrelated to yield components, then such traits may improve grain plumpness without having deleterious effect on yield or malt-quality related traits.

Consensus mapping studies increase our understanding of the complexity and association between grain quality traits and agronomic traits in barley. In the V × B mapping population, all genomic regions significantly associated with the grain texture traits, endosperm hardness and grain density, were coincident with QTLs for grain size, grain yield, and/or flowering date, with the exception of the one region detected on chromosome 6H (Fig. 2). Vernalisation genes, photoperiod genes, and barley cellulose synthase-like genes were coincident with grain density QTLs on chromosome 2H, and endosperm hardness QTLs on chromosomes 4H, 5H, and 7H. As genes and their functionalities are identified along the barley genome, so to will our understanding of how agronomic traits, such as grain yield, flowering date, and plant development genes, interact with grain texture, size and malt-quality related QTLs.

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