Theor Appl Genet (2013) 126:1873–1884
DOI 10.1007/s00122-013-2102-0

ORIGINAL PAPER

QTL dissection of the loss of green colour during post-anthesis grain maturation in two-rowed barley

Livinus C. Emebiri

Received: 26 November 2012 / Accepted: 10 April 2013 / Published online: 19 April 2013

Abstract Ability to genetically manipulate the loss of green colour during grain maturation has potentials for increasing productivity, disease resistance, and drought and heat tolerance in crop plants. Two doubled haploid, two-rowed barley populations (Vlamingh × Buloke and VB9524 × ND11231*12) were monitored over 2 years for loss of green colour during grain filling using a portable active sensor. The aims were to determine the genomic regions that control trait heritability by quantitative trait locus (QTL) analysis, and to examine patterns of QTL-environment interactions under different conditions of water stress. In the Vlamingh × Buloke cross, broad-sense heritability estimate for loss of green colour (measured as the difference in sensor readings taken at anthesis and maturity, ΔSRI) was 0.68, and 0.78 for the VB9524 × ND11231*12 population. In the VB9524 × ND11231*12 population, rapid loss of green colour was positively associated with grain yield and percent plump grains, but in the Vlamingh × Buloke population, a slower loss of green colour (low ΔSRI) was associated with increased grain plumpness. With the aid of a dense array of single nucleotide polymorphisms (SNPs) and EST-derived SSR markers, a total of nine QTLs were detected across the two populations. Of these, a single major locus on the short arm of barley chromosome 5H was consistently linked with trait variation across the populations and multiple environments. The QTL was independent of flowering time and explained between 5.4 and 15.4 % of the variation observed in both populations, depending on the environment, and although a QTL × E interaction was detected, it was largely due to a change in the magnitude of the effect, rather than a change in direction. The results suggest that loss of green colour during grain maturation may be under the control of a simple genetic architecture, but a careful study of target populations and environments would be required for breeding purposes.

Communicated by T. Komatsuda.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-013-2102-0) contains supplementary material, which is available to authorized users.

L. C. Emebiri (✉)
EH Graham Centre for Agricultural Innovation (Industry and Investment NSW and Charles Sturt University), Wagga Wagga Agricultural Institute, Wagga Wagga, NSW 2650, Australia
e-mail: Livinus.Emebiri@dpi.nsw.gov.au

Introduction

Leaves of many crop species tend to senesce and abscise during seed development, as manifested by loss of green colour. The main purpose is the mobilisation and recycling of nutrients, from the leaves to the developing seeds to prepare for the next generation (Diaz et al. 2005). The importance to crop productivity cannot be over-emphasised, as it overlays and is tightly integrated with the plant nutrient uptake efficiency, grain yield, lodging resistance and disease resistance (Borrell et al. 2000; WenJiang et al. 2007; Blake et al. 2007; Gregersen et al. 2008; Zheng et al. 2008; Joshi et al. 2007). In oats, the maintenance of a large photosynthetically active system longer after flowering was implicated in the development of larger kernels (Helsel and Frey 1978).

While the importance, from a practical perspective, is widely recognised, breeding crop cultivars for characteristics related to senescence is not an easy task. Although leaf yellowing is often conspicuous in the field, documenting the process is difficult, as observed by

Abdelkhalik et al. (2005). At the basic level, this can be accomplished by visual observations, which, although reliable, is subjective and, therefore, difficult to reproduce by other researchers (Inada 1985; Adamsen et al. 1999; Lim et al. 2007). It can also be monitored using optical sensors, such as a SPAD-502 meter, which is sensitive to photosynthetic pigments of individual leaves. The SPAD-502 meter measures the amount of chlorophyll in the leaf, and the results correlate linearly with extractable chlorophyll concentrations (Arunyanark et al. 2008). However, its use for characterising the senescence of entire canopies is somewhat limited because, in practice, leaf selection for SPAD measurement is subject to operator bias (Adamsen et al. 1999). The SPAD meter measures only one spot of one leaf on each measurement, so a large number of random observations must be averaged to reduce variability and make statistical comparisons between treatments meaningful. The use of the meter is also time-consuming and laborious since a specific leaf on a small plant must be located and placed in the meter with each measurement (Murdock et al. 2004). The results are often inconsistent (Joshi et al. 2007), and there are also concerns that repeated measurements on the same leaves over time may affect their physiological status because of the physical contact with the leaf (Adamsen et al. 1999).

More recently, portable handheld optical sensors have been developed that offer the twin advantages of increased speed and reduced effort to make each reading and also a canopy measurement rather than a single leaf measurement. The spectral reflectance in the visible (VIS) wavelengths (400–700 nm) depends on the absorption of light by leaf chlorophyll and associated pigments such as carotenoids and anthocyanins. The reflectance in the VIS is low because of the high absorption of light energy by pigments. The reflectance in the near-infrared (NIR) wavelengths (700–1,300 nm) is high because of the multiple scattering of light by different leaf tissues (Knipling, 1970). Measurements in the VIS and NIR regions are easily converted into indices, amongst which the most widespread are the simple ratio, SR [SR = NIR/VIS] and the normalised difference vegetative index, NDVI [NDVI = (NIR − VIS)/(NIR + VIS)] (Araus et al. 2002). By periodic measurement during crop growth, spectral reflectance may be of particular use in obtaining an objective measure of senescence (Araus et al. 2008; Foulkes et al. 2007).

In this study, two doubled haploid barley populations were monitored over 2 years for loss of green colour during grain filling using a portable active sensor. The aims of this paper were to determine the genomic regions that control trait heritability by quantitative trait locus (QTL) analysis, and examine patterns of QTL-environment interactions under conditions of water stress. Genetic linkage mapping was accomplished using a dense array of SNPs and EST-derived SSR markers, which enabled inferences on putative candidate genes.

Materials and methods

Mapping populations

The first population comprised 351 doubled haploid (DH) lines derived from the Vlamingh × Buloke cross, a population developed for the genetic dissection of grain plumpness (Moody et al. 2009). Both Vlamingh (WABAR0570/TR118) and Buloke (Franklin/VB9104//VB9104) are tall, spring commercial malting barley varieties with similar phenology but contrast both in terms of regions of adaptation and pedigree. The second population consisted of 180 DH lines derived from the VB9524 × ND11231*12 cross (Emebiri et al. 2003). VB9524 is an advanced selection two-row line derived from a cross between Arapiles and Franklin, while ND11231*12 is a two-row barley line derived from crosses involving Karl, a six-rowed low-protein variety (Burger et al. 1979), as an ancestral parent.

Field trials and phenotyping

Field trials of both populations were grown at the DPI Victoria's Plant Breeding Centre, near Horsham (36°43′S, 142°12′E), Victoria, Australia. The trials were sown on the 15th of June, 2007 and the 18th of June 2008. Both the DH populations were grown in 2007, but due to logistic reasons, only the Vlamingh × Buloke population was sown in 2008. Other entries in the trials include Dash, Binalong, Baudin, Gairdner, Sloop, Flagship, Hindmarsh, VB0325, Barque, Franklin and Schooner. These are commercial feed and malting quality varieties (except VB0325), selected to represent different adaptations to low and high-rainfall environments. All entries were grown under irrigated and water-stressed conditions in both years, with irrigation water provided by flooding, which supplied each plot with 25 mm of water over a 24-h period. Growing-season rainfall data obtained during the period are presented in supplementary Table 1. Plots measured 6 m in length at sowing, with six rows of plants spaced 15 cm apart, at a seeding rate of 40 g per plot or 50 kg ha^{-1}. The trials were maintained free of weeds and leaf diseases by chemical applications.

The field trials were monitored for loss of green colour using a handheld optical sensor. The sensor (Crop Circle model ACS-210, Holland Scientific, USA) collected ten readings per second with 40–50 values acquired from each

Table 1 Phenotypic correlation between ΔSRI and agronomic traits measured under dryland and irrigated conditions for two mapping populations of two-rowed barley

Traits	Dryland	Irrigated
Vlamingh × Buloke ($N = 351$)		
Grain yield	0.02 ns	−0.06 ns
Grain size (1,000 GW)	0.11*	−0.10 ns
Plump grain (>2.5 mm)	−0.20***	−0.15**
Plant height	0.10 ns	0.06 ns
Flowering time	0.07 ns	0.21***
VB9524 × ND11231*12 ($N = 180$)		
Grain yield	0.21**	0.46***
Grain size (1,000 GW)	−0.07 ns	0.06 ns
Plump grain (>2.5 mm)	0.28***	0.25**
Plant height	−0.02 ns	−0.04 ns
Flowering time	−0.08 ns	−0.19**

ns correlation is not significant

*, **, *** Significant correlation at $P < 0.05$, $P < 0.01$ and $P < 0.001$

plot, depending on the speed of walking. Readings were taken twice during the growth cycle, first at the time of 50 % awn emergence, and the second at physiological maturity, with the meter held about 30–45 cm from the canopy at 90° angle of the meter in relation to the canopy surface. All measurements recorded for a single plot were averaged to determine a mean value. Two wavebands of information in the VIS and NIR portions of the spectrum were used to calculate a simple ratio index (SRI) as suggested by Inada (1985):

$$\mathrm{SRI} = \frac{\mathrm{NIR}}{\mathrm{VIS}}.$$

Loss of green colour was quantified as the relative difference in SRI readings taken at anthesis and at physiological maturity, and designated ΔSRI. In addition, data were recorded on flowering time, plant height, grain yield per plot, grain size and plumpness. Flowering time was measured as the number of days from sowing to emergence of awn from the boot on 50 % of the plants in a plot; plant height was measured at maturity as the distance from the soil surface to the top of the main spike (awns excluded) in five plants per plot. For grain yield, each plot was first trimmed to about 5 m in length before machine harvesting. A sub-sample was retained from each harvested plot to measure grain size and plumpness. Grain size was determined from the weight of 100 grains taken from unscreened samples, and expressed as the weight of 1,000 grains. Grain plumpness was measured by agitating a 100 g sample on a Sortimat (Pfeuffer GmbH, Kitzingen, Germany) machine for 1 min and collecting the fractions retained above a 2.5 mm screen.

Statistical analysis

All data were subjected to analysis of variation, using the method of restricted maximum likelihood (REML) as implemented in GENSTAT 15 (VSN International 2012). For each trait in each environment, the data were subjected to spatial analysis to account for field heterogeneity, using spatial information of the plot layouts (rows and columns) and modelling trends using the separable autoregressive (AR) process (AR1 × AR1) proposed by Cullis and Gleeson (1991). First, the analysis was carried out with genotype fitted as a random factor, to assess the proportion of variance accounted for by genotype and to subsequently calculate broad-sense heritability. Then, the data were re-analysed with all terms (replicates and genotypes) considered as fixed, to produce the best linear unbiased estimates (BLUEs), which were used for QTL mapping. The final model, for loss of green colour, included flowering time as a covariate, but this term was dropped from the model when its effect was found not significant.

Genetic linkage map construction and QTL analysis

The Vlamingh × Buloke DH population was genotyped with a combination of SNP, SSR and EST-SSR markers. For SNP marker assay, DNA extracted from parents and the doubled haploid progeny were sent to the Southern California Genotyping Consortium, Illumina BeadLab at the University of California, Los Angeles (UCLA) for the OPA-SNP assay with BOPA1, a 1536-plex SNP detection platform (Close et al. 2009). In addition, the population was screened with 75 SSR and EST-SSR markers, selected from the markers described by Emebiri (2009) on the basis of providing good genome coverage and polymorphism between the parental varieties.

The VB9524 × ND11231*12 cross was genotyped with SSR and AFLP markers. It was updated during the course of this study by replacing some of the AFLP markers with newly developed EST-derived SSR markers reported by Emebiri (2009).

Individual maps were constructed for both populations *de novo*, using MultiPoint software (http://www.multiqtl.com/), which implements the Evolutionary Strategy Algorithm of Mester et al. (2003). Linkage map construction was performed as described in the tutorials, with multilocus orders based on 500 bootstrap iterations to detect and remove unreliable marker loci. In the Vlamingh × Buloke population, 1,409 of the 1,536 SNP loci were of good quality (call frequency >90 %), and from these, 480 polymorphic SNPs were used for genetic linkage analyses. Altogether, 540 polymorphic markers were used, and the linkage maps varied in density from 38 markers on chromosome 1H to 117 markers on 5H. The total map

length was 2,059.1 cM, varying from 180.9 cM on chromosome 6H to 387.3 cM on chromosome 7H (Supplementary Material 2). The genetic linkage map of VB9524 × ND11231*12 was constructed with 249 markers, and varied in density from 30 markers on chromosome 1H to 53 markers on 7H (Supplementary Material 3).

The GENSTAT package (15th edition) was used for QTL analyses, in which we used linear mixed models to run a series of analyses for QTL main effect and QTL × E interactions. After selecting a suitable variance–covariance structure (uniform covariance with unequal variance), the initial analyses involved scanning the genome for evidence of QTLs using a simple-interval mapping approach to fit single-QTL models every 10 cM along the genome. In the second step, we tested for QTLs at particular positions after correcting for QTLs elsewhere in the genome (Composite Interval Mapping), with the minimum cofactor proximity set at 50 cM. In the third step, we first included in the model all significant QTLs obtained from the previous step as a candidate set of QTLs and then performed backward selection based on the Wald F statistic. The genome-wide significance level of $\alpha = 0.05$ $[-\log_{10}(0.05) = 1.3]$ for declaring a significant QTL was established by the Li and Ji (2005) method. To compare results, data were also analysed using an approach implemented in MultiQTL software, in which a combined multi-interval (MIM) and multi-environment (QTL × E) analyses were applied (Korol et al. 2001; Rae et al. 2008). MultiQTL software was also used to calculate power of QTL detection by running bootstrap analyses.

Results

Phenotypic variation

A graphical display of genetic variation in mean values for ΔSRI is presented in Fig. 1. The results showed that, irrespective of growing conditions, the varieties could be roughly grouped into those with accelerated versus slow loss of green colour after flowering. Varieties with rapid loss of green colour included Franklin, Flagship and Gairdner, while Vlamingh, Dash, ND11231*12, Hindmarsh and its sister line VB0325 were placed on the opposite quadrangle. Under water-stressed conditions, the varieties Barque, VB9524 and Schooner exhibited a faster loss of green colour, as indicated by the higher ΔSRI values, while Dash, Vlamingh and the sister lines Hindmarsh and VB0325 had markedly lower ΔSRI values, indicating they retained green colour longer after flowering.

Of the mapping parents, Vlamingh and Buloke had very similar flowering dates, an average of 274 in julian days for Vlamingh and 275 days for Buloke. The grain filling

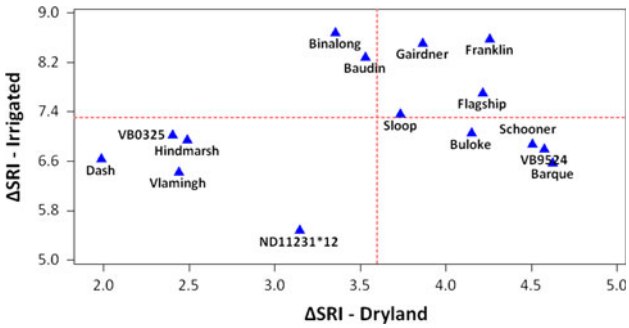

Fig. 1 Graphical display of average ΔSRI values for parent and checks used for trials conducted in the 2007 and 2008 under dryland and irrigated conditions. *Small values* indicate early senescence and high values stay-greenness

durations were also similar, as reported by Moody et al. (2009), an average of 37 days for Vlamingh and 40 days for Buloke. However, the two parents were significantly different in their ability to retain green leaf colour after anthesis (Fig. 1). The differences were more pronounced when grown under water-stressed, dryland conditions than under conditions of supplemental irrigation (Fig. 2). To illustrate, under dryland conditions, Vlamingh had a smaller ΔSRI value (average = 2.4 ± 0.6) than Buloke (average = 3.5 ± 0.8), indicating it retained green leaf colour a bit longer after anthesis than Buloke. However, in the irrigated trials, Vlamingh (6.4 ± 0.02) and Buloke (6.8 ± 0.03) had similar ΔSRI values, indicating no difference in green leaf area retention.

In the VB9524 × ND11231*12 population, the parents were also significantly different in their ability to retain leaf green colour (Fig. 1). Furthermore, the differences were consistent across environments (Fig. 2), with the loss of green colour being more rapid in VB9524 than in ND11231*12.

Genetic variation amongst doubled haploids

Statistical tests for genotypic variation in ΔSRI were highly significant in both populations, indicating the existence of genetic control. The ratio of genetic to phenotypic variation (heritability) for ΔSRI in the Vlamingh × Buloke cross was 0.68, and 0.78 for the VB9524 × ND11231*12 population, suggesting a strong genetic basis for loss of green colour during grain maturation in these populations. The statistical tests for genotype-by-environment (G × E) interactions, however, had variable results, being highly significant ($P < 0.001$) in the VB9524 × ND11231*12 population and not significant (P = 0.43) in the Vlamingh × Buloke population. In the VB9524 × ND11231*12 population, the significant G × E was largely due to heterogeneity of variances (scaling effect), which does not alter the ranking of the genotypes (that is, it

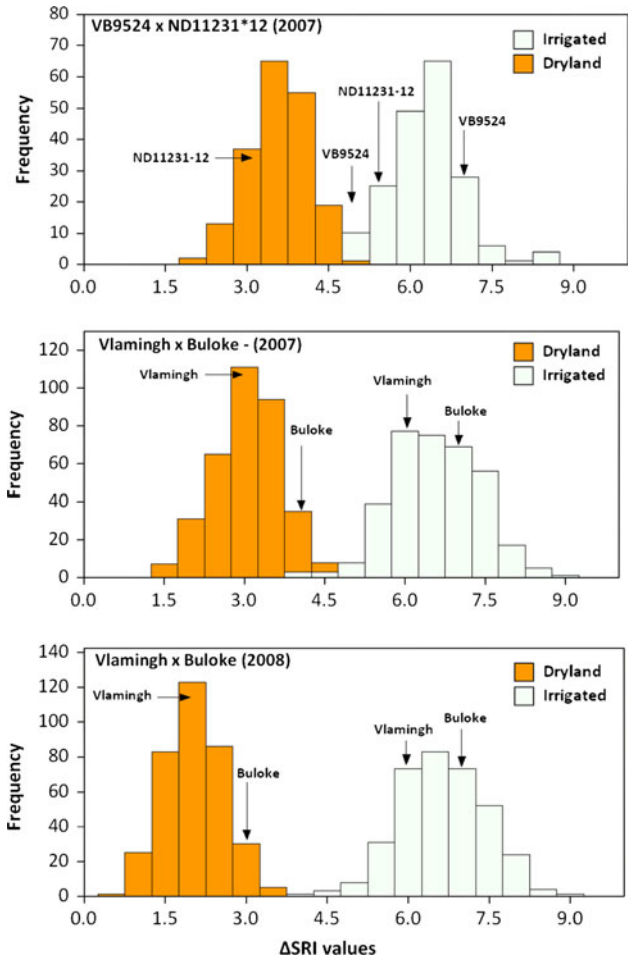

Fig. 2 Frequency distributions of loss of green colour measured as ΔSRI during post-anthesis grain development in the two doubled haploid populations. The data were averages for 2007 and 2008 years, in trials grown under dryland (*filled bars*) and irrigated (*un-filled bars*) conditions

is the non-crossover type G × E interaction). As shown in Fig. 2, the ΔSRI values were much larger under irrigation (average ΔSRI = 6.5 ± 0.03) than in water-stressed conditions (average ΔSRI = 2.9 ± 0.03), but the genotypes maintained their individual rankings, as illustrated for parents. Frequency distribution of phenotypes in both populations followed a pattern that is consistent with quantitative traits (Fig. 2), with mean trait values for many of the doubled haploid lines falling outside the mean value for either of the parents.

Association with agronomic traits

Phenotypic correlation of ΔSRI with a range of agronomic traits was examined in the two barley crosses. The most consistently significant ($P \leq 0.05$) associations were those with grain plumpness (Table 1). However, the directions of the associations were different, being positive in the VB9524 × ND11231*12 population, and negative in the Vlamingh × Buloke cross. The association with grain yield was positive and consistently significant in the VB9524 × ND11231*12 population, but not in the Vlamingh × Buloke cross. In both populations, ΔSRI showed little or no significant association with plant height and kernel weight (1,000 GW), and although it was significantly associated with earliness under irrigated conditions, the directions were different (Table 1).

QTL analysis

At a genome-wide false discovery rate of 0.05, the MultiQTL and GENSTAT mapping approaches identified the same significant loci on the genome of Vlamingh × Buloke population (Table 2; Fig. 3), with powers ranging from 94 to 100 %. The results for the VB9524 × ND11231*12 population were also very similar, but with one exception, i.e., an additional QTL on the short arm of chromosome 7H detected by the mixed model analysis with GENSTAT software (Table 2; Fig. 3). For the sake of brevity, therefore, only the results based on the mixed model analysis are presented.

In both Vlamingh × Buloke and VB9524 × ND11231*12 populations, strong and consistent QTLs were detected on chromosomes 5H, at the 5H_bin06 map position (Table 2; Fig. 3). The QTL explained between 5.4 and 15.4 % of the variation observed in both populations, depending on the environment, and although a QTL × E interaction was detected, it was largely due to a change in the magnitude of the effect, rather than a change in direction (Fig. 3, lower panel). In both crosses, a QTL was detected on chromosome 2H, but at slightly different bin-map positions (Table 2). Particularly evident in the Vlamingh × Buloke population, this QTL exhibited significant crossover type QTL × E interactions, i.e., allele effects depended on the environment, being positive under dryland, and negative in the irrigated trials (Table 2; Fig. 3). In the VB9524 × ND11231*12 population, the QTL was detected only under dryland conditions, explaining 9.0 % of the phenotypic variation. Although the loci on the chromosomes 4H and 7HL were population-specific, they were consistently expressed in all environments, i.e., the superior allele coming from a particular parent was strongly active in all environments.

Multi-trait QTL analysis

Given the physiological nature of ΔSRI, it was then desired to test for pleiotropy or close linkage to the QTLs influencing other agronomic traits, such as grain yield, grain size and flowering time. For this purpose, a multi-trait QTL mapping approach was applied, in which variation for

Table 2 Descriptive summary results from a mixed linear, single-QTL model

Chr.	−log10 (*P* value)	SNP locus	Bin	QTL × E	Dryland 2007		Irrigated 2007		Dryland 2008		Irrigated 2008	
					PEV	Add. effect	PEV	Add. effect	PEV	Add. effect	PEV	Add. effect
Vlamingh × Buloke												
1H	4.79	1_0552	1H_bin09	Yes	2.5	−0.10**	0.4	0.05 ns	3.2	−0.05***	0.7	0.07 ns
2H	4.42	1_1100	2H_bin10	Yes	3.6	0.12***	1.4	−0.09*	0.4	0.02 ns	0.4	−0.05 ns
4H	4.66	2_0020	4H_bin07	No	0.9	0.06***	0.6	0.06***	3.8	0.06***	0.5	0.06***
5H	18.41	2_0306	5H_bin06	Yes	15.4	−0.24***	0.2	−0.03 ns	9.2	−0.09***	5.4	−0.18***
6H	3.86	2_0835	6H_bin07	Yes	3.9	−0.12**	1.8	0.10*	2.2	−0.04*	1.8	−0.10*
ND11231*12 × VB9524												
2H	4.53	EBmac850	2H_bin08	Yes	9.0	0.16***	0.3	0.03 ns	NA	NA	NA	NA
5H	6.65	P11M47-122	5H_bin06	Yes	8.4	−0.15***	9.2	−0.19***	NA	NA	NA	NA
7HS	3.16	ABC158	7H_bin05	Yes	0.3	−0.03 ns	6.2	−0.15***	NA	NA	NA	NA
7HL	7.10	Bmag135	7H_bin14	Yes	5.5	−0.12***	11.4	−0.21***	NA	NA	NA	NA

Results presented are for chromosomes with significant QTLs in the Vlamingh × Buloke and ND11231*12 × VB9524 populations

ns the effect is not significant, *NA* not available

*, **, *** Significant effect at $P < 0.05$, $P < 0.01$ and $P < 0.001$

several agronomic traits was analysed simultaneously. The results presented in Fig. 4 showed that, with a few exceptions, all QTLs identified in the single-trait analyses were still significant in the multi-trait model (Fig. 4), thus placing very high level of confidence in these QTL. The notable exceptions were in the Vlamingh × Buloke population, where the ΔSRI QTL on chromosome 1H and 6H was no longer significant in the multi-trait model (Fig. 4), and the QTL on 2H was shown to co-locate with flowering time QTL (Table 3). The multi-trait QTL model identified additional QTL that was not identified with the single-trait model, specifically on chromosome 3H and on 7HS in the Vlamingh × Buloke population. The QTL on 7HS was detected in the VB9524 × ND11231*12 population (Figs. 3, 4), and in both populations, the QTL was strongly associated with flowering time (DAE).

In both populations, there was little effect of flowering time QTLs at the 5HS QTL region associated with ΔSRI (Fig. 4; Tables 3, 4). Rather, the QTL region was significantly associated with grain plumpness (both populations), and to grain size in (Vlamingh × Buloke cross). In the Vlamingh × Buloke population, Buloke alleles at the 5HS region were associated with rapid loss of green colour (high-value ΔSRI phenotype), and increased grain size (Table 3). In the VB9524 × ND11231*12 cross, the VB9524 parent contributed the alleles for high-value ΔSRI phenotype, and also alleles that could increase grain plumpness. These results suggest that selection for favourable alleles at the ΔSRI loci would have favourable pleiotropic effects on improving other agronomic traits.

Discussions

The loss of green colour during leaf senescence is one of the most manifest natural phenomena (Hörtensteiner and Kräutler 2000), but it is very hard to phenotype, objectively. While previous studies have used visual ratings to phenotype senescence at a given point in time (e.g. Rae et al. 2006), or multiple times during grain filling (e.g. Verma et al. 2004; Vijayalakshmi et al. 2010; Bogard et al. 2011), inherent subjectivity of the technique renders synchronising results between different researchers virtually impossible (Xu et al. 2000). Here, an optical sensor was used to measure loss of green colour during grain filling in two different populations. As sensor readings were taken at the same time for all genotypes, this was invariably a measure of the rate of senescence, rather than the onset or duration of senescence. Phenotypic correlations of the loss of green colour (measured as changes in SRI, ΔSRI) with agronomic traits were different across the two populations (Table 1). In the VB9524 × ND11231*12 population, rapid loss of green colour was positively associated with grain yield and percent plump grains, but in the Vlamingh × Buloke population, a slower loss of green colour (low ΔSRI) was associated with increased grain plumpness.

The delayed onset of leaf senescence (stay-green) is more widely described in the literature and has been proven to increase yield (Borrell et al. 2000; Bekavac et al. 2007; Zheng et al. 2008; Lopes and Reynold 2012; Bogard et al. 2011) due to a longer period of active photosynthesis (Thomas and Howarth 2000). However, it can increase

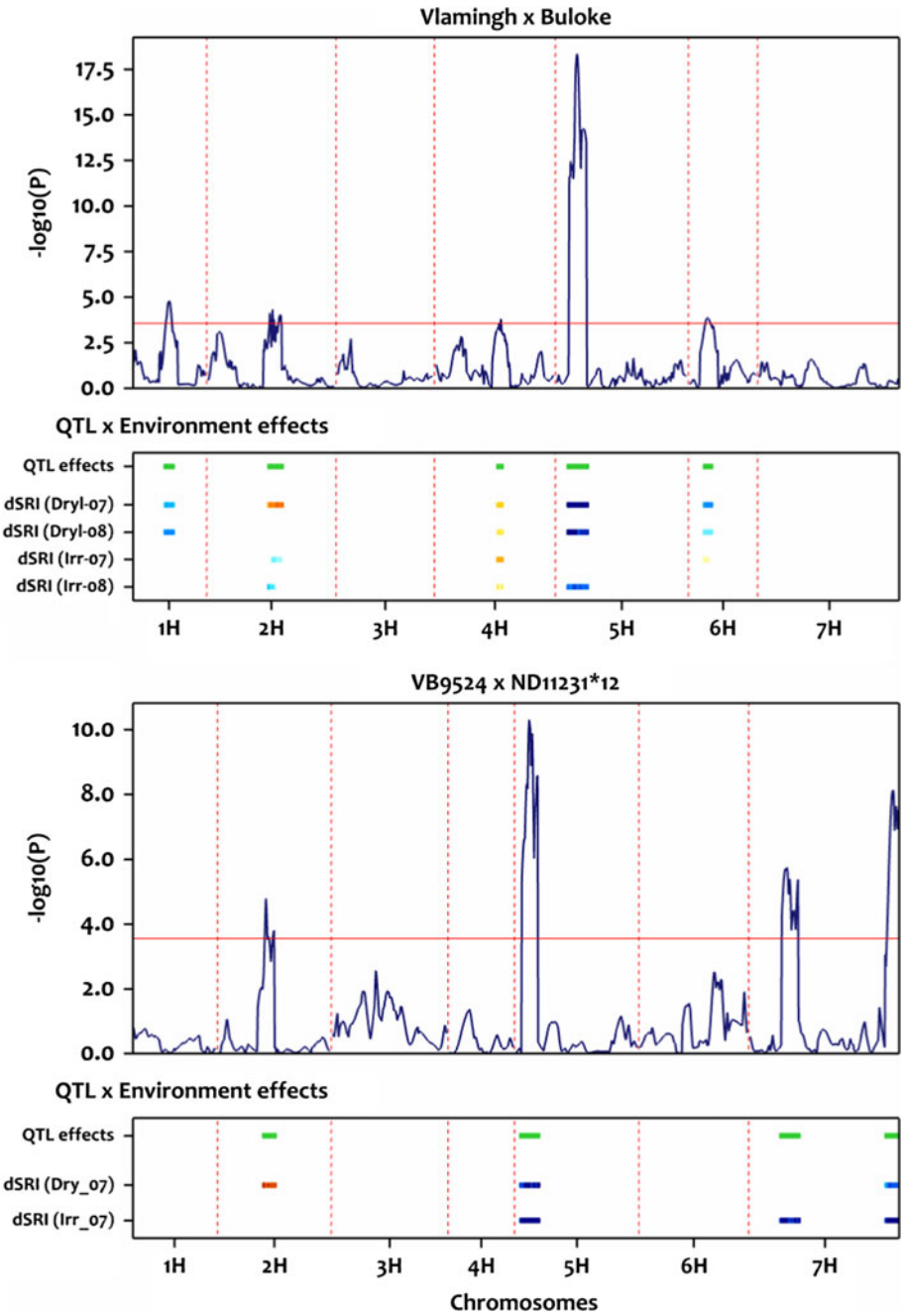

Fig. 3 QTL profiles of Vlamingh × Buloke and the VB9524 × ND11231*12 populations, showing chromosomal regions with significant QTLs (*upper panel*) and their additive effect across environments (*lower panel*). The image files were generated from GENSTAT 14th edition software, by running an initial scan (SIM) and then a scan with cofactors (the composite interval mapping model). *dSRI* refers to ΔSRI measured under dryland (*Dry*) or irrigated (*irr*) conditions in 2007 and 2008

vulnerability to terminal heat stress and drought if the rate of senescence is inherently slow, as the overriding stress factor will impose accelerated senescence or leaf kill (Blum 1998; Gregersen et al. 2008). Rapid rate of senescence, on the other hand, confers efficient nutrient remobilisation and can lead to increased yield, but so far, little is known about the category of genes that control the rate of leaf senescence (Wu et al. 2012).

Flowering time-independent QTL

A single major locus on the short arm of barley chromosome 5H was found to be consistently linked with trait variation across the populations and multiple environments (Tables 2, 3, 4; Figs. 3, 4). The QTL remained highly significant even after adjusting for the confounding effect of flowering time using a multi-trait QTL model (Fig. 4;

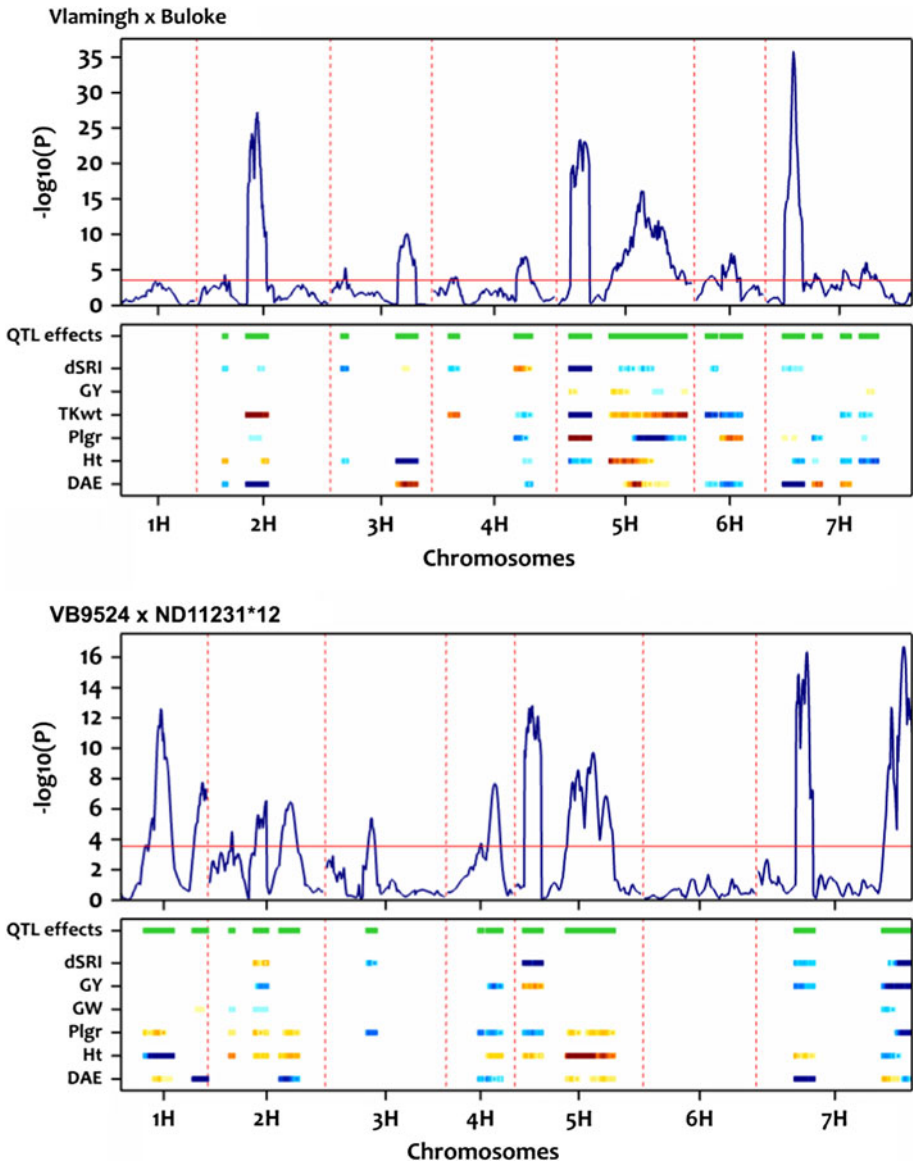

Fig. 4 Multi-trait QTL profiles of the Vlamingh × Buloke and VB9524 × ND11231*12 populations, showing chromosomal regions with significant QTLs (*upper panel*) and their additive effects (*lower panel*). The image files were generated from GENSTAT 15th edition, after running an initial scan (SIM) and then a scan with cofactors (the composite interval mapping model). *dSRI* loss of green colour (measured as ΔSRI), *GY* grain yield, *GW* 1,000 grain weight, *Plgr* percent grain plumpness, *Ht* plant height, while DAE refers to the number of days from sowing to awn emergence

Tables 3, 4), suggesting that the locus affected rate of senescence in a manner independent of flowering time. There were QTLs for heading date on chromosome 5H in both populations (Fig. 4), but these were detected on the long arm, possibly corresponding to the *earliness per se* gene, eps5HL (Emebiri and Moody 2006).

The genomic location of the QTL on 5HS QTL (bin06) corresponds to positions of QTL linked to chlorophyll fluorescence parameters (Guo et al. 2008), grain protein content, kernel plumpness (Emebiri et al. 2003; 2005; Moralejo et al. 2004), and increased rate of grain filling (Moody et al. 2009). It also coincides with the locus identified by Mickelson et al. (2003) that influenced total leaf nitrogen at mid-grain fill and differences in leaf nitrogen content between anthesis and maturity (Yang et al. 2004), which supports the functional relationship between leaf senescence and nitrogen metabolism. Sinclair and de Wit (1975) have proposed that leaf area loss after flowering might be a response to the nitrogen demand of growing seeds. It is, therefore, conceivable to suggest that, at the 5HS locus, the gene underlying the loss of green colour might be a gene that influences nitrogen metabolism, and hence would be an attractive target for improving nitrogen-use efficiency.

Comparison of populations

In the VB9524 × ND11231*12 population, most of the identified QTL were independent of flowering time, with the exception of chromosome 7H QTLs (Fig. 3; Table 4). In this population, ΔSRI was positively correlated to grain yield and grain plumpness (Table 1), a result that may

Table 3 QTL parameters derived from running a multi-trait QTL model in GENSTAT 15th edition

Traits	Add. effect	High-value allele parent	SE	*P* value	PEV
QTL (locus name): 1_0436 − Chr. 2H; [−log(*P*) = 27.25]					
ΔSRI	0.030	Buloke	0.020	0.131	0.6
Grain yield	0.002	Vlamingh	0.005	0.750	0.0
1,000 GW	0.450	Vlamingh	0.057	0.000	14.1
Plump grains	0.585	Buloke	0.326	0.072	0.9
Plant height	0.101	Vlamingh	0.249	0.684	0.0
Flowering time	0.643	Buloke	0.091	0.000	9.5
QTL (Locus name): 2_0612 − Chr. 3H; [−log(*P*) = 11.12]					
ΔSRI	0.043	Vlamingh	0.022	0.049	1.2
Grain yield	0.005	Vlamingh	0.005	0.378	0.3
1, 000 GW	0.039	Buloke	0.062	0.527	0.1
Plump grains	0.254	Buloke	0.355	0.474	0.2
Plant height	1.714	Buloke	0.271	0.000	13.0
Flowering time	0.470	Vlamingh	0.099	0.000	5.1
QTL (locus name): 2_0732 − Chr. 4H; [−log(*P*) = 6.92]					
ΔSRI	0.067	Vlamingh	0.020	0.001	3.0
Grain yield	0.007	Vlamingh	0.005	0.167	0.6
1,000 GW	0.148	Buloke	0.056	0.008	1.5
Plump grains	0.798	Buloke	0.320	0.013	1.6
Plant height	0.507	Buloke	0.244	0.038	1.1
Flowering time	0.153	Buloke	0.089	0.085	0.5
QTL (locus name): 2_0306 − Chr. 5H; [−log(*P*) = 23.37]					
ΔSRI	0.137	Buloke	0.021	0.000	12.7
Grain yield	0.001	Vlamingh	0.005	0.821	0.0
1,000 GW	0.431	Buloke	0.059	0.000	12.9
Plump grains	1.823	Vlamingh	0.337	0.000	8.6
Plant height	0.677	Buloke	0.258	0.009	2.0
Flowering time	0.009	Vlamingh	0.094	0.926	0.0
QTL (locus name): 1_0124 − Chr. 6H [−log(*P*) = 7.38]					
ΔSRI	0.013	Buloke	0.019	0.502	0.1
Grain yield	0.003	Buloke	0.005	0.521	0.1
1,000 GW	0.205	Buloke	0.055	0.000	2.9
Plump grains	1.317	Vlamingh	0.314	0.000	4.5
Plant height	0.381	Vlamingh	0.240	0.112	0.6
Flowering time	0.355	Buloke	0.087	0.000	2.9
QTL (locus name): 2_0126 − Chr. 7H [−log(*P*) = 35.79]					
ΔSRI	0.044	Buloke	0.020	0.026	1.3
Grain yield	0.002	Vlamingh	0.005	0.710	0.0
1,000 GW	0.092	Buloke	0.056	0.099	0.6
Plump grains	0.625	Vlamingh	0.321	0.052	1.0
Plant height	0.467	Buloke	0.246	0.057	1.0
Flowering time	1.147	Buloke	0.089	0.000	30.2

The parameters for the Vlamingh × Buloke population were obtained by backward selection, after running an initial scan (SIM) and then a scan with cofactors (the composite interval mapping model)

appear contradictory, since photosynthetic proteins such as Rubisco are usually degraded early during leaf senescence (Feller and Fischer 1994), leading to decrease in photosynthetic rates (Mickelson et al. 2003). Photosynthesis during the grain filling accounts for 75 % of the final grain carbon content, with the remaining 25 % made up of re-mobilised storage carbohydrates (stem reserves) laid down before anthesis (Yoshida 1981). Blum (1998) reported that wheat genotypes with high capacity for stem reserve utilisation for grain filling had accelerated leaf senescence

Table 4 QTL parameters derived from running a multi-trait QTL model in GENSTAT 15th edition

Traits	Add. effect	High-value allele parent	SE	*P* value	PEV
QTL (locus name): EBmac850 − Chr. 2H; [−log(*P*) = 5.62]					
ΔSRI	0.093	ND11231*12	0.029	0.002	3.6
Grain yield	0.053	VB9524	0.025	0.031	2.0
1,000 GW	0.117	VB9524	0.055	0.033	2.6
Plump grains	0.577	ND11231*12	0.193	0.003	3.9
Plant height	1.015	ND11231*12	0.399	0.011	3.4
Flowering time	0.049	ND11231*12	0.121	0.686	0.1
QTL (locus name): Bmac67 − Chr. 3H; [−log(*P*) = 5.42]					
ΔSRI	0.112	VB9524	0.031	0.000	5.3
Grain yield	0.030	VB9524	0.026	0.244	0.6
1,000 GW	0.096	ND11231*12	0.058	0.097	1.8
Plump grains	0.826	VB9524	0.204	0.000	8.0
Plant height	0.692	ND11231*12	0.422	0.102	1.6
Flowering time	0.010	ND11231*12	0.128	0.938	0.0
QTL (locus name): P11M47-122 − Chr. 5H; [−log(*P*) = 11.96]					
ΔSRI	0.167	VB9524	0.031	0.000	11.6
Grain yield	0.077	ND11231*12	0.025	0.002	4.2
1,000 GW	0.018	VB9524	0.057	0.756	0.1
Plump grains	0.627	VB9524	0.199	0.002	4.6
Plant height	1.296	ND11231*12	0.414	0.002	5.5
Flowering time	0.001	ND11231*12	0.126	0.991	0.0
QTL (locus name): ABC158 − Chr. 7HS; [−log(*P*) = 12.90]					
ΔSRI	0.088	VB9524	0.032	0.005	3.2
Grain yield	0.098	VB9524	0.026	0.000	6.8
1,000 GW	0.050	ND11231*12	0.059	0.392	0.5
Plump grains	0.099	ND11231*12	0.206	0.629	0.1
Plant height	0.964	ND11231*12	0.427	0.024	3.0
Flowering time	0.848	VB9524	0.130	0.000	22.5
QTL (locus name): Bmag135 − Chr. 7HL; [−log(*P*) = 15.82]					
ΔSRI	0.167	VB9524	0.030	0.000	11.6
Grain yield	0.143	VB9524	0.025	0.000	14.5
1,000 GW	0.085	VB9524	0.055	0.122	1.4
Plump grains	0.926	VB9524	0.194	0.000	10.0
Plant height	0.084	ND11231*12	0.401	0.834	0.0
Flowering time	0.325	VB9524	0.122	0.008	3.3

The parameters for the VB9524 × ND11231*12 population were obtained by backward selection, after running an initial scan (SIM) and then a scan with cofactors (the composite interval mapping model)

under both stress and non-stress conditions. This seems to be linked to accelerated export of nitrogen from leaves (Blum 1998), and it is conceivable, therefore, to suggest that, in the VB9524 × ND11231*12 population, genotypes compensated for the reduction in photosynthesis during grain filling by inducing greater stem reserve mobilisation, and utilisation by the grain. An interesting follow-up study would be to characterise this population for water soluble carbohydrates, and its relevance to abiotic stress tolerance.

With the exception of the 5HS region, all other QTLs identified in the Vlamingh × Buloke population were linked to flowering time (Fig. 3; Table 3). The QTL for ΔSRI on chromosome 2H was localised in the same region harbouring the *Ppd-H1* locus, the major determinant of photoperiod response, and on chromosome 3H, the QTL was co-localised with the earliness per se locus, *esp3HL* and the semi-dwarfing gene, *sdw1*. The 3H region was an additional locus identified in both populations, after running a multi-trait QTL model. It was significantly linked to flowering time in the Vlamingh × Buloke population, but not in the VB9524 × ND11231*12 population (Tables 3, 4).

The study also identified a QTL on chromosome 6H in the Vlamingh × Buloke population, but not in the VB9524 × ND11231*12 population (Fig. 3; Table 2). The evidence for this QTL was not very strong after multi-trait

QTL analysis (Table 3), indicating it might be influenced by genes controlling other agronomic traits. This QTL was of interest because it was located within the chromosomal region that harbours a QTL for grain protein in six-rowed barley (See et al. 2002). The barley grain protein content QTL on chromosome 6H is colinear with the *Gpc-B1* gene on wheat chromosome 6B (Distelfeld et al. 2008), and in both wheat and barley, this QTL is known to influence flowering time (Lacerenza et al. 2010; Parrott et al. 2012) and leaf senescence (Heidlebaugh et al. 2008; Jukanti et al. 2008; Parrott et al. 2012; Uauy et al. 2006a, b).

Another QTL on the short arm of chromosome 7H also co-localised with the flowering time locus, *Vrn-H3* gene (Wang et al. 2010). In all cases, the allelic effect is such that late flowering induces accelerated leaf senescence, which is consistent with the observed positive correlation between ΔSRI and flowering time (Table 1). The results suggest that, in this population, flowering time might be an intrinsic factor in speeding up the rate of senescence. As a slow rate of leaf senescence was associated with increased grain plumpness, this would require early flowering and a prolonged period of grain filling to optimise yield. This population would be useful for improving the 'stay-green' capacity, but the trait may not be ideal for abiotic stress tolerance in environments that experience stress during grain filling.

In summary, the findings in this study suggest that, in two-rowed barley, the loss of green colour during grain maturation may be under the control of a simple genetic architecture. This comprised a major locus on chromosome 5HS that is independent of flowering time and a number of minor loci with small effects that are influenced by flowering time. The flowering time-independence and large effects of the 5HS QTL suggest genetic manipulation by breeding would be worthwhile.

Acknowledgments This work was supported by Grants from the Grains Research and Development Corporation (GRDC), under project DAV00082.

References

Abdelkhalik AF, Nomura RSK, Ikehashi H (2005) QTL-based analysis of leaf senescence in an indica/japonica hybrid in rice (*Oryza sativa* L.). Theor Appl Genet 110:1226–1235

Adamsen FJ, Pinter PJ, Barnes EM, LaMorte RL, Wall GW, Leavitt SW, Kimball BA (1999) Measuring wheat senescence with a digital camera. Crop Sci 39:719–724

Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C3 cereals: what should we breed for? Ann. Bot. (Lond.) 89:925–940

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. Crit Rev Plant Sci 27:377–412

Arunyanark A, Jogloy S, Akkasaeng C, Vorasoot N, Kesmala T, Nageswara Rao RC, Wright GC, Patanothai A (2008) Chlorophyll stability is an indicator of drought tolerance in Peanut. J Agron Crop Sci 194:113–125

Bekavac G, Purar B, Stojakovic M, DJ Jockovic, Ivanovic M, Nastasic A (2007) Genetic analysis of stay-green trait in broad-based maize populations. Cereal Res Commun 35:31–41

Blake NK, Lanning SP, Martin JM, Sherman JD, Talbert LE (2007) Relationship of flag leaf characteristics to economically important traits in two spring wheat crosses. Crop Sci 47:491–496

Blum A (1998) Improving wheat grain filling under stress by stem reserve mobilisation. Euphytica 100:77–83

Bogard M, Jourdan M, Allard V, Martre P, Perretant MR, Ravel C, Heumez E, Orford S, Snape J, Griffiths S, Gaju O, Foulkes J, Le Gouis J (2011) Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. J Exp Bot 62:3621–3636

Borrell AK, Hammer GL, Henzell RG (2000) Does maintaining the green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. Crop Sci 40:1037–1048

Burger WC, Wesenberg DM, Carden JE, Pawlisch PE (1979) Protein concentration and composition of Karl and related barleys. Crop Sci 19:235–238

Close TJ et al (2009) Development and implementation of high-throughput SNP genotyping in barley. BMC Genomics 10:582

Cullis BR, Gleeson AC (1991) Spatial analysis of field experiments—an extension to two dimensions. Biometrics 47:1449–1460

Diaz C, Purdy S, Christ A, Morot-Gaudry JF, Wingler A, Masclaux-Daubresse C (2005) Characterization of markers to determine the extent and variability of leaf senescence in Arabidopsis. A metabolic profiling approach. Plant Physiol 138:898–908

Distelfeld A, Korol A, Dubcovsky J, Uauy C, Blake T, Fahima T (2008) Colinearity between the barley grain protein content (GPC) QTL on chromosome arm 6HS and the wheat Gpc-B1 region. Mol Breed 22:25–38

Emebiri LC (2009) EST-SSR markers derived from an elite barley cultivar (*Hordeum vulgare* L. 'Morex'): polymorphism and genetic marker potential. Genome 52(8):665–676

Emebiri LC, Moody DB (2006) Heritable basis for some genotype–environment stability statistics: inferences from QTL analysis of heading date in two-rowed barley. Field Crops Res 96:243–251

Emebiri LC, Moody D, Panozzo J, Chalmers K, Kretschmer J, Ablett G (2003) Identification of QTLs associated with variations in grain protein concentration in two-row barley. Aust J Agric Res 54:1211–1221

Emebiri LC, Moody DB, Horsley R, Panozzo J, Read BJ (2005) The genetic control of grain protein content variation in a doubled haploid population derived from a cross between Australian and North American two-rowed barley lines. J Cereal Sci 41:107–114

Feller U, Fischer A (1994) Nitrogen metabolism in senescing leaves. Crit Rev Plant Sci 13:241–273

Foulkes MJ, Sylvester-Bradley R, Weightman R, Snape JW (2007) Identifying physiological traits associated with improved drought resistance in winter wheat. Field Crops Res 103:11–24

Gregersen PL, Holm PB, Krupinska K (2008) Leaf senescence and nutrient remobilisation in barley and wheat. Plant Biol 10:37–49

Guo P, Baum M, Varshney RK, Graner A, Grando S, Ceccarelli S (2008) QTLs for chlorophyll and chlorophyll fluorescence parameters in barley under post-flowering drought. Euphytica 163:203–214

Heidlebaugh NM, Trethewey BR, Jukanti AK, Parrott DL, Martin JM, Fischer AM (2008) Effects of a barley (*Hordeum vulgare*) chromosome 6 grain protein content locus on whole-plant nitrogen reallocation under two different fertilisation regimes. Funct Plant Biol 35:619–632

Helsel DB, Frey KJ (1978) Grain yield variations in Oats associated with differences in leaf area duration among Oat lines. Crop Sci 18:765–769

Hörtensteiner S, Kräutler B (2000) Chlorophyll breakdown in oilseed rape. Photosynth Res 64:137–146

Inada K (1985) Spectral ratio of reflectance for estimating chlorophyll content of leaves. Japan J Crop Sci 54:261–265

Jiang GH, He YQ, Xu CG, Li XH, Zhang Q (2004) The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an indica by japonica cross. Theor Appl Genet 108:688–698

Joshi AK, Kumari M, Singh VP, Reddy CM, Kumar S, Rane J, Chand R (2007) Stay green trait: variation, inheritance and its association with spot blotch resistance in spring wheat (*Triticum aestivum* L.). Euphytica 153:59–71

Jukanti AK, Heidlebaugh NM, Parrott DL, Fischer IA, McInnerney K, Fischer AM (2008) Comparative transcriptome profiling of near-isogenic barley (*Hordeum vulgare*) lines differing in the allelic state of a major grain protein content locus identifies genes with possible roles in leaf senescence and nitrogen reallocation. New Phytol 177:333–349

Knipling EB (1970) Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. Remote Sensing of Environment 1:155–159

Korol AB, Ronin YI, Itskovich AM, Peng J, Nevo E (2001) Enhanced efficiency of quantitative trait loci mapping analysis based on multivariate complexes of quantitative traits. Genetics 157:1789–1803

Lacerenza JA, Parrott DL, Fischer AM (2010) A major grain protein content locus on barley (*Hordeum vulgare* L.) chromosome 6 influences flowering time and sequential leaf senescence. J Exp Bot 61:3137–3149

Li J, Ji L (2005) Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity 95(3):221–227

Lim PO, Kim HJ, Nam HG (2007) Leaf senescence. Annu Rev Plant Biol 58:115–136

Lopes MS, Reynolds MP (2012) Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology. J Exp Bot 63:3789–3798

Mester D, Ronin Y, Minkov D, Nevo E, Korol A (2003) Constructing large-scale genetic maps using an evolutionary strategy algorithm. Genetics 165:2269–2282

Mickelson S, See D, Meyer FD, Garner JP, Foster CR, Blake TK, Fischer AM (2003) Mapping of QTL associated with nitrogen storage and remobilization in barley (*Hordeum vulgare* L.) leaves. J Exp Bot 54:801–812

Moody DB, Tait S, Black C, Panozzo JP, Emebiri LC, Hayden MJ, Steadman E (2009) Genetic dissection of grain plumpness in barley: alternative approaches and inter-species comparisons. In: Abstract of paper presented at the 14th Australasian plant breeding and 11th SABRAO Conference, 10–14 August, Cairns-Queensland, Australia

Moralejo M, Swanston JS, Muñoz P, Prada D, Elía M, Russell JR, Ramsay L, Cistué L, Codesal P, Casas AM, Romagosa I, Powell W, Molina-Cano JL (2004) Use of new EST markers to elucidate the genetic differences in grain protein content between European and North American two-rowed malting barleys. Theor Appl Genet 110:116–125

Murdock L, Call D, James J (2004) Comparison and use of chlorophyll meters on wheat (reflectance vs. transmittance/absorbance). Cooperative extension service, University of Kentucky-College of Agriculture, AGR-181

Parrott DL, Downs EP, Fischer AM (2012) Control of barley (*Hordeum vulgare* L.) development and senescence by the interaction between a chromosome six grain protein content locus, day length, and vernalization. J Exp Bot 63:1329–1339

Rae AM, Ferris R, Tallis MJ, Taylor G (2006) Elucidating genomic regions determining enhanced leaf growth and delayed senescence in elevated CO_2. Plant Cell Environ 26:1730–1741

Rae AM, Pinel MPC, Bastien C, Sabatti M, Street NR, Tucker J, Dixon C, Marron N, Dillen SY, Taylor G (2008) QTL for yield in bioenergy *Populus* : identifying G × E interactions from growth at three contrasting sites. Tree Genet Genomes 4:97–112

See D, Kanazin V, Kephart K, Blake T (2002) Mapping genes controlling variation in barley grain protein concentration. Crop Sci 42:680–685

Sinclair TR, de Wit CT (1975) Comparative analysis of photosynthate and nitrogen requirements in the production of seeds of various crops. Science 189:565–567

Thomas H, Howarth CJ (2000) Five ways to stay green. J Exp Bot 51:329–337

Uauy C, Brevis JC, Dubcovsky J (2006a) The high grain protein content gene Gpc-B1 accelerates senescence and has pleiotropic effects on protein content in wheat. J Exp Bot 57:2785–2794

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006b) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314:1298–1301

Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, Snape JW (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135:255–263

Vijayalakshmi K, Fritz A, Paulsen G, Bai G, Pandravada S, Gill B (2010) Modeling and mapping QTL for senescence-related traits in winter wheat under high temperature. Mol Breed 26:163–175

VSN International (2012) GenStat for Windows, 15th edition. VSN International, Hemel Hempstead, UK. http://www.GenStat.co.uk

Wang G, Schmalenbach I, von Korff M, Leon J, Kilian B, Rode J, Pillen K (2010) Association of barley photoperiod and vernalization genes with QTLs for flowering time and agronomic traits in a BC2DH population and a set of wild barley introgression lines. Theor Appl Genet 120:1559–1574

WenJiang H, Lamb DW, Zheng N, YongJiang Z, LiangYun L, JiHua W (2007) Identification of yellow rust in wheat using in situ spectral reflectance measurements and airborne hyperspectral imaging. Precis Agric 8:187–197

Wu X-Y, Kuai B-K, Jia J-Z, Jing H-C (2012) Regulation of leaf senescence and crop genetic improvement. J Integr Plant Biol 54:936–952

Xu W, Rosenow DT, Nguyen HT (2000) Stay-green trait in grain sorghum: relationship between visual rating and leaf chlorophyll concentration. Plant Breed 119:365–367

Yang L, Mickelson S, See D, Blake TK, Fischer AM (2004) Genetic analysis of the function of major leaf proteases in barley (*Hordeum vulgare* L.) nitrogen remobilization. J Exp Bot 55:2607–2616

Yoshida S (1981) Physiological analysis of rice yield. In: Fundamentals of rice crop science. International Rice Research Institute, Makita City, pp 231–251

Zheng HJ, Wu A, Zheng CC, Wang YF, Cai R, Shen XF, Xu RR, Liu P, Kong LJ, Dong ST (2008) QTL mapping of maize (*Zea mays*) stay-green traits and their relationship to yield. Plant Breed 128:54–62