Identification of quantitative trait loci contributing to yield and seed quality parameters under terminal drought in barley advanced backcross lines

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Abstract Drought is a major limiting factor for barley production, especially in the primary areas of its cultivation. Wild barley represents a major source of favourable alleles for increasing the genetic variation for multiple traits including resistance to both biotic and abiotic stresses. We used advanced backcross quantitative trait locus (AB-QTL) analysis of a BC3-doubled haploid population developed between the cultivated parent Brenda (*Hordeum vulgare* ssp. *vulgare*) and the wild accession HS584 (*H. vulgare* ssp. *spontaneum*) to study the contribution of wild barley in improving various agronomic and seed quality traits under post-anthesis drought. The experiment was carried out at two different locations (IPK, Gatersleben and Nordsaat, Böhnshausen) and terminal drought was imposed by withholding water or spraying with potassium iodide at 10 days after flowering under greenhouse or field conditions, respectively. QTL analysis indicated that wild barley contributed favourably to most of the traits studied under both control and drought conditions. A total of seven hotspot QTL regions with co-localizing QTL for various traits harboured more than 80 % of the stable QTL detected in the present study. For yield and thousand-grain weight and their respective drought tolerance indices, most of the QTL were derived from Brenda. On the other hand, for traits like seed length and seed nitrogen content, all the QTL were contributed by HS584, the parent having higher trait value. A significantly reduced carbon/nitrogen (C/N) ratio in the selected contrasting inferior lines compared to superior ones suggests that C/N ratio could be a potential parameter for screening not just seed quality parameters but also grain weight performance under terminal drought.

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Introduction

Among various environmental factors, drought has devastating effects on crop production worldwide. In the face of increasing global climatic changes, its intensity and frequency is expected to increase, especially in arid and semi-arid regions (Ceccarelli

et al. 2010; Fereres et al. 2011). In cereals under drought stress, a major concern is reduction of grain yield and impaired quality, the severity of which depends on the particular stage of plant development at which drought occurs (Dolferus et al. 2011). Barley is often cultivated under rainfed conditions and generally encounters drought during the grain-filling period, which is called terminal or post-anthesis drought (Blum 2009).

Anthesis is one of the most crucial stages of crop development and is severely affected by drought in terms of yield (Dolferus et al. 2011). When drought occurs during anthesis, it reduces fertility and hence seed number which in turn reduces grain yield, whereas drought occurrence during early seed development reduces grain yield mainly through reduction in grain weight (Jamieson et al. 1995; Barnabas et al. 2008).

Owing to the complexity of drought (intensity, duration and time of occurrence) and crop responses to drought, development of drought-tolerant varieties lags far behind compared to other traits (Yang et al. 2010; Sinclair 2011). However, recent advances made in various fields such as plant phenotyping, functional genomics, quantitative trait locus (QTL) mapping and crop physiology have enabled the identification of the key genes and networks mediating the yield response under drought (Mir et al. 2012; Tuberosa and Salvi 2006).

A pre-requisite for any successful crop breeding programme is the genetic diversity available for crop improvement. However, the genetic diversity available in most of the present cultivated crops, including barley, is limited due to selection processes mainly targeted towards yield under favourable conditions (Harlan 1976; Tanksley et al. 1996). This is well exemplified in the findings of Ellis et al. (2000), who reported that only 40 % of the wild alleles are found in cultivated barley. Hence, it has become imperative for breeders to look for favourable alleles in wild ancestors of a particular crop species and transfer them to cultivated varieties to broaden their performance under different challenging environments (Tanksley and McCouch 1997). Although wild species are inferior to cultivated varieties for a number of agronomic traits, various studies have showed that about 30–50 % of the favourable alleles in breeding lines were contributed by wild relatives (Xiao et al. 1998; Thomson et al. 2003; Pillen et al. 2003; Frary

et al. 2004; Von Korff et al. 2006; Swamy and Sarla 2008; Nevo and Chen 2010).

Many QTL studies carried out using wild barley as a donor parent for various traits indicated that it is a potential source for trait improvement (Nevo 1992; Volis et al. 2000; Pillen et al. 2004; Li et al. 2005, 2006; Rostoks et al. 2005; Schmalenbach et al. 2009; Schnaithmann and Pillen 2013). In addition, *H. vulgare* ssp. *spontaneum* was also found to possess positive alleles for abiotic stresses such as drought and salt (Talame et al. 2004; Suprunova et al. 2007; Ceccarelli et al. 2007; Lakew et al. 2011, 2013). Major hindrances to the utilization of wild species in crop improvement using conventional breeding are the quantitative nature of most of the agronomic traits and the linkage drag of undesirable genes present in wild species (Wang and Chee 2010). One of the breeding strategies to overcome the problem of linkage drag associated with wild genotypes during breeding programmes is advanced backcross quantitative trait locus (AB-QTL) analysis, which combines QTL detection with the introduction of favourable alleles into the targeted variety (Tanksley and Nelson 1996). In barley, AB-QTL analysis was first reported by Pillen et al. (2003) using a BC2F2 population developed between the cultivar Apex and the wild accession ISR101-23 for various agronomic and malting quality traits. Some of the other studies for improving drought tolerance in barley include Baum et al. (2003), Ceccarelli et al. (2004), Forster et al. (1997), Grando et al. (2001) and Ivandic et al. (2003).

While advanced backcrossing together with molecular markers is an efficient means of using wild species for crop improvement through QTL analysis, the development of introgression line (IL) libraries and the development of near isogenic lines are further steps in QTL analysis, validation and cloning. IL libraries represent the entire genome of a wild species in a genetic background of the cultivated parent, with each line carrying one or few segments of the wild genome segments (Zamir 2001). They are especially important in studying complex traits, as the effect of small/single introgression of a wild genome can be studied in a uniform background of the elite cultivar thus overcoming the problem of large phenotypic variation and the gene interactions between the donor alleles. In addition, ILs are also useful in breeding and in identifying genes through map-based cloning (Yano 2001; Fonceka et al. 2012). IL libraries are developed in many crops

including tomato, rice, lettuce, wheat, barley etc., for various traits including yield under drought conditions in rice (Xu et al. 2005; Schmalenbach et al. 2009; Röder et al. 2008; Fonceka et al. 2012). The first IL set in barley was reported by Schmalenbach et al. (2008, 2009), and has been recently refined using SNP markers (Schmalenbach et al. 2011).

The present study was undertaken to assess the importance of wild barley in contributing to terminal drought tolerance in terms of both yield and seed quality, using an advanced backcross line (AB line) population consisting of 67 BC3-doubled haploid (DH) lines developed using the German spring barley cultivar, Brenda (*Hordeum vulgare* ssp. *vulgare*) and the wild accession, HS584 (*Hordeum vulgare* ssp. *spontaneum*). In addition, we also analyzed various seed morphological traits such as seed length, breadth and shape, which are the sub-components of thousand-grain weight (TGW).

Materials and methods

Plant material

A panel of 67 AB lines (BC3-DH) derived from a cross between German spring barley cultivar Brenda (*Hordeum vulgare* ssp. *vulgare*) as the recurrent parent and the wild accession HS584 (*H. vulgare* ssp. *spontaneum*) as the donor parent was used for this study. A BC3 population of the same cross was earlier analyzed for wild barley's contribution to various yield components and disease resistance traits by Li et al. (2006). The subset of population used in the present study (67 lines) represents the introgression of the whole HS584 genome at various positions in the genetic background of Brenda which were selected from an earlier population of 120 lines. A graphical representation of these 67 BC3-DHs with introgressions of HS584 segments at various positions in the genetic background of Brenda is given as Supplemental Figure S1.

Experimental locations and drought treatment

Experiments were conducted at two different locations in Germany, one at a plant breeding station, Nordsaat GmbH (Böhnshausen), and another at IPK, Gatersleben during 2007–2009 under both greenhouse and field conditions. At Nordsaat (2007 and 2008),

terminal drought was imposed in the field by spraying with potassium iodide (KI; 0.4 %) and in the greenhouse by withholding water, at 8 DAF (days after flowering). In the field, all genotypes were planted as three-row plots (0.5 m²) per entry with two replications in randomized blocks, whereas in the greenhouse they were grown as two-row plots per entry with two replications in randomized blocks. Under both conditions (field and greenhouse), drought was imposed at 8 DAF for the drought treatment group, whereas control plants continued to be irrigated until seed maturity.

At IPK, the experiments were carried out during 2008 and 2009 under greenhouse and field conditions, respectively. The details of the various traits scored under each environment are given in Supplemental Table S1. At IPK greenhouse, plants were grown in individual pots with a 16-h light/20 °C and 8-h dark/15 °C cycle. Irrigation was achieved through an automatic irrigation system (Delta T devices) that consisted of a large number of soil moisture sensors (SM200) connected to a central device (DL2 data logger) that is capable of recording, storing soil moisture content and irrigating according to the conditions defined. Control plants were maintained at 40 % soil moisture content (100 % field capacity) from sowing until maturity, whereas stress plants were maintained at 10–15 % (25–30 % field capacity) from 8 DAF to maturity. Supplemental Figure S2 is a representative example for soil moisture content maintained during the experiment. Each genotype had 10 plants per treatment. Under field conditions in IPK, plants were grown only under control condition with a similar design as in Nordsaat.

Phenotyping

Yield

Except at Nordsaat field (2008), where yield was calculated on a per plot basis, for all other experiments yield was calculated from a group of 10 plants per treatment when the plants had reached their physiological maturity.

Thousand grain weight (TGW)

The average weight of 1,000 grains was calculated from 10 plants per plot using a digital seed analyzer (Marvin; GTA Sensorik GmbH, Germany).

Drought tolerance index for yield and TGW (Fischer and Maurer 1978):

Drought tolerance index (DTI) was calculated as

$$DTI = (Gc \times Gds)/(Xc)^2$$

where Gc = TGW under control, Gds = TGW under drought, Xc = mean TGW of all genotypes under control.

Seed morphological traits

Seed length and breadth were measured from the seeds harvested from the respective treatments using the digital seed analyzer, Marvin. Seed shape (length to breadth ratio) was calculated from the primary data obtained.

Seed starch, carbon and nitrogen content

Seed starch content was measured as mentioned in Worch et al. (2011). Seed C and N% was measured in dry powdered samples using an elemental analyzer (vario EL *III*; Elementaranalysesysteme GmbH Hanau, Germany).

Genotyping

Genotyping of the BC3-DH lines was carried out using a set of simple sequence repeats (SSR) and single nucleotide polymorphisms (SNPs). A total of 145 markers were genotyped on the present BC3-DH population. The mapping positions were adopted from the map of Worch et al. (2011). Taking the genetic map of barley to be 1,071 cM (Worch et al. 2011), on average a marker was obtained every 7.3 cM. Details of genotyping using SSRs and SNPs can be found in Li et al. (2006) and Worch et al. (2011), respectively.

QTL analysis and statistics

QTL analysis and correlation between the traits were carried out using Qgene 3.0 software (Nelson 1997). QTL regions were identified based on the linkage map developed by Worch et al. (2011) using various expressed sequence tag (EST)-based SNPs and SSR markers. Markers and their positions on the chromosomes are given in Supplemental Table S2. QTL analysis was performed for control and stress treatments

separately for each environment. To determine the effect of a particular marker on each trait, initially a single marker regression was carried out and the markers lying close to each other were considered to be in a QTL region. The regions of the genome were identified as QTL only if a particular marker/QTL appeared in at least two environments with an LOD ≥3.0 for yield, TGW and their tolerance indices. For other traits [seed length, breadth, length to breadth ratio (L/B), seed starch amount per seed and nitrogen content], a QTL was considered if it had an LOD ≥3.0 and appeared in at least one treatment (control or drought), as these traits were recorded only in one environment (IPK, Gatersleben). The relative performance (RP) of HS584 QTL compared to Brenda alleles for various phenotypic traits was estimated using RP = (BB − AA)/AA, where BB is the phenotypic mean for the individuals with HS584 alleles and AA is the phenotypic mean for the individuals with Brenda alleles at a particular locus (Pillen et al. 2003). QTL regions were represented on a consensus linkage map developed using EST-based SNP markers based on three populations (Worch et al. 2011). Significant differences between the treatment groups (control and drought) were carried out using Student's *t* test in Microsoft Office Excel 2003 and the significance levels are indicated by *($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$).

Results

Descriptive statistics and correlations among traits

There was a significant variation in the different traits measured and they were generally normally distributed. Trait statistics such as mean, minimum, maximum and coefficient of variation show that transgressive segregation was visible in yield, DTI-yield, TGW and DTI-TGW, whereas for other traits data for HS584 were missing (Supplemental Tables S3 and S4). In general, all trait values were higher under control than stress except for seed nitrogen content which was higher under stress.

Correlations of different traits within an environment are summarized for the Nordsaat and IPK experiments in Supplemental Tables S5 and S6, respectively. In most of the environments, yield and TGW under control were significantly correlated to their respective traits under stress, indicating that

relative ranking of the genotypes remained similar under both conditions. Interestingly, yield and TGW were significantly correlated in all the greenhouse experiments but not in any of the field experiments. This is probably due to the effect of KI spray on grain weight and hence yield. Similarly, drought tolerance indices for yield and TGW were significantly correlated ($P < 0.001$) with their respective traits under control and stress conditions in all environments. Starch being the predominant form of storage compound in barley, a very strong positive correlation was observed between starch amount per seed and TGW under both control (0.82) and drought conditions (0.87). Among the seed morphological traits, seed breadth was positively correlated with both starch amount per seed and TGW under both conditions. On the other hand, while a negative trend was observed for seed breadth with seed nitrogen content, seed length and nitrogen content were positively correlated (Supplemental Tables S6). Seed L/B, which indicates the seed shape, was negatively correlated to TGW. In cereals, there is an apparent negative correlation of yield and TGW with seed nitrogen content, which was also evident in this study.

Correlation of a particular trait across different environments is an indication of trait stability (Supplemental Tables S7 to S10). Of all the traits scored, TGW had the highest and most significant correlations between different environments, which is consistent with the high heritability of TGW in cereals. In addition, a strong positive correlation (0.70) in DTI-yield was observed between Nordsaat field and greenhouse conditions in 2008.

Consistent QTL across the environments (stable QTL)

QTL analysis of traits for each environment and treatment (control and drought/KI) was carried out separately. Considering all environments, a total of 124 QTL were detected for different traits; however, only those which appeared in more than one environment (in the case of yield and TGW and its DTI) were considered for further data interpretation and analysis.

Grain yield (qYLD)

There were altogether five stable QTL obtained for yield; of them three were mapped to chromosome 1H

and one each to chromosomes 2H and 3H. The most stable qYLD was qYLD1.3, which appeared in four environments, followed by qYLD1.1 and qYLD1.2, which were detected in three environments; all the three QTL above were expressed under both control and drought conditions. The qYLD2.1 and qYLD3.1 were found to be control- and drought-specific, respectively, and the latter was the only QTL contributed by the wild parent, HS584 (Supplemental Table S11 and Fig. 1). The QTL explaining most of the phenotypic variations for yield were found on chromosome 1H (qYLD1.1 and qYLD1.3) and 2H (qYLD2.1); the presence of HS584 segments at these regions had significantly reduced yield under both conditions.

Drought tolerance index for yield (qDTI-Y)

A total of four stable QTL were obtained for DTI-Y that mapped to chromosomes 1H (three) and 2H (one). The most stable QTL were qDTI-Y1.2 and qDTI-Y1.1, which appeared in four and three environments, respectively. All qDTI-Y were contributed by Brenda except qDTI-Y1.3 which appeared in two environments (Supplemental Table S11; Fig. 1).

Thousand-grain weight (qTGW)

Among all traits, the highest numbers of stable QTL were detected for TGW (11), which mapped to all seven linkage groups except chromosomes 4H and 5H. Most of the qTGW were derived from Brenda except for qTGW3.1 and qTGW3.2. The most stable QTL, qTGW1.1, qTGW1.3 and qTGW2.1, were all detected under both treatments. qTGW3.2 derived from HS584 and qTGW7.2 derived from Brenda were found to be specific to control and drought conditions, respectively (Supplemental Table S12; Fig. 1).

Drought tolerance index for thousand-grain weight (qDTI-GW)

A total of five QTL were detected for DTI-TGW that mapped to chromosomes 1H (three), 2H (one) and 6H (one), and were all derived from Brenda. Of these, the most stable was qDTI-GW6.1, which appeared in four environments (Supplemental Table S12; Fig. 1).
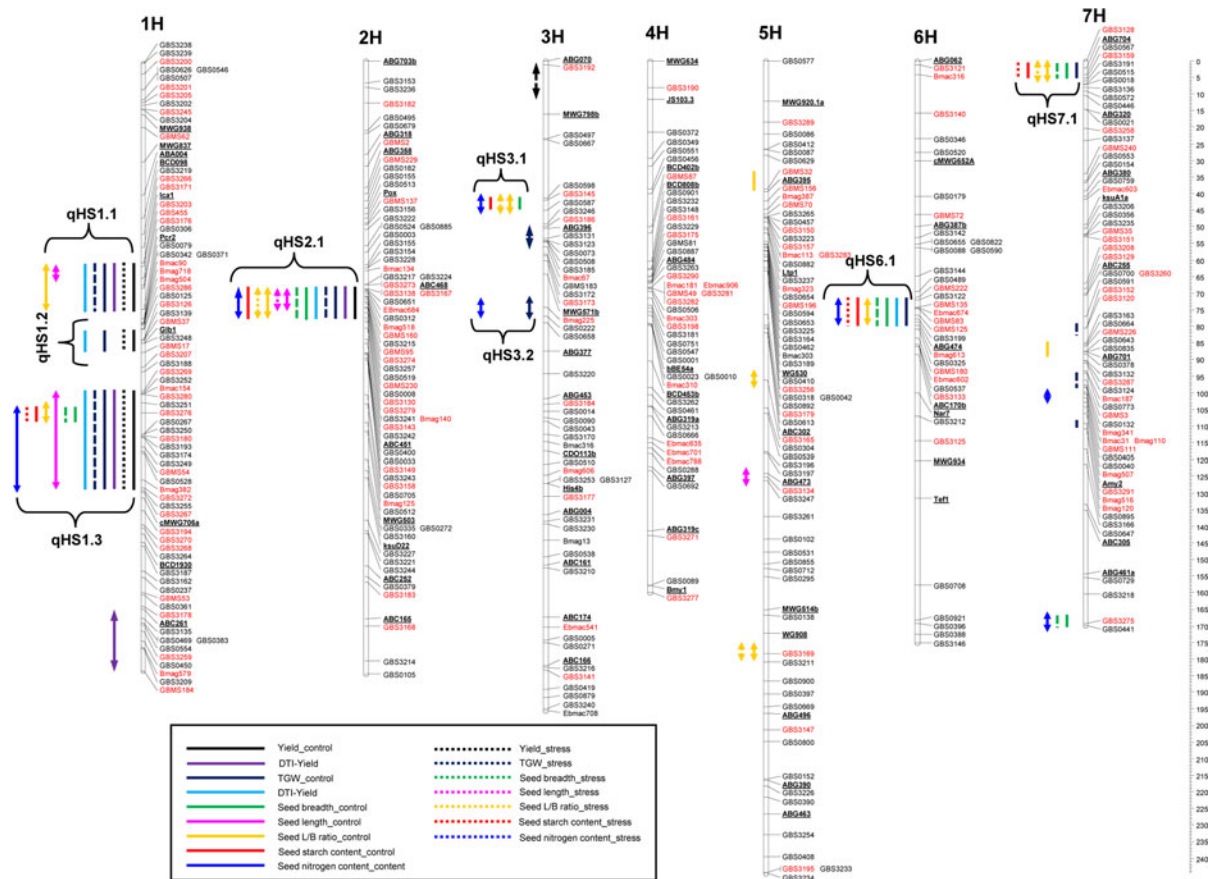
Fig. 1 QTL identified for various traits under control and drought conditions in the BC3-DH population derived from the cross Brenda/HS584. QTL contributed by HS584 are indicated by *arrowheads*. qHS refers to hot-spot QTL. The linkage map was adopted from Worch et al. (2011) and the markers in *red* and *black* are the ones which are polymorphic in the Brenda/HS584

population and another two populations (Streptoe/Morex and Oregon/Wolf), respectively. Phenotypic traits are indicated by *different colours* and *solid straight and dotted lines of a colour* indicate a particular trait under control and drought conditions, respectively

QTL analysis of grain quality and seed characteristics

Here we focused on seed morphological traits (length, breadth and L/B) and grain composition (starch and nitrogen) from the phenotype data collected by growing the plants under more controlled conditions at IPK greenhouse and including technical replications (Supplemental Table S8). There was a large variation in both the morphological as well as the quality traits studied within the BC3-DH population. Parents represented the extreme values for various morphological traits, indicating that HS584 and Brenda possessed all negative and positive alleles, respectively, for these

traits except seed breadth (Supplemental Figure S3). Within the DH population, seed length ranged from 9.8 mm in DH19 to 12.1 mm in DH5 (Supplemental Figure S3A), whereas seed breadth ranged from 3.7 mm in DH58 to 4.4 mm in DH60 (Supplemental Figure S3B). Seed L/B ranged from 2.33 in DH19 to 2.96 in DH5 (Supplemental Figure S3C). A large set of AB lines such as DH7, DH42, DH60, DH22 and DH23 with similar or lower grain width than Brenda had significantly higher TGW as a result of increased seed length. This is a clear indication of positive effect of grain length on TGW. Within the DH population, starch content ranged from 8.2 mg/seed in DH50 to 33.9 mg/seed in DH9 under drought while seed N%

ranged from 1.25 % in DH37 to 2.98 % in DH57 (Supplemental Figures S4A and B). Interestingly, in the extreme drought-susceptible DHs, we noticed that reduction in yield, TGW and starch content was accompanied by a significant increase in seed nitrogen content.

Seed breadth (qSB)

For seed breadth, a total of six QTL were detected that mapped to all seven linkage groups except chromosomes 4H and 5H. Interestingly, all seed breadth QTL were contributed by Brenda and three QTL, qSB3.1, qSB6.1 and qSB7.2, were specific to drought, while others were detected under both conditions (Supplemental Table S13 and Fig. 1).

Seed length (qSL)

A total of four QTL were detected for seed length that mapped to chromosomes 1H (two), 2H (one) and 5H (one). The only QTL which was common to both conditions was qSL2.1 and the rest were control-specific. All qSL were contributed by HS584 which had the higher value for this trait among the parents (Supplemental Table S13; Fig. 1).

Seed length to breadth ratio (qSLB)

A total of ten QTL were detected for seed L/B that mapped to all seven linkage groups except 4H. The two QTL derived from Brenda (qSLB5.1 and qSLB7.2) were found to be control-specific. Of the HS584-derived QTL, qSLB1.1, qSLB1.2, qSLB5.2 and qSLB6.1 were found to be control-specific and the rest were present under both conditions. No QTL was found to be drought-specific (Supplemental Table S13; Fig. 1).

Seed starch content (qSS)

A total of five QTL were detected for starch amount per seed that mapped one each to chromosomes 1H, 2H, 3H, 6H and 7H. Two QTL, qSS2.1 and qSS3.1, were specific to control and the rest were present in both conditions. All qSS were derived from Brenda and those with the highest significance (LOD) were qSS2.1 and qSS7.1, which were specific to control and

drought conditions, respectively (Supplemental Table S14 and Fig. 1).

Seed nitrogen content (qSN)

For seed nitrogen content, a total of seven QTL were detected that mapped one each to chromosomes 1H, 2H and 6H and two each to 3H and 7H. Of all the QTL, qSN7.1 and qSN3.2 were the only drought-specific ones, while the rest were control-specific; no QTL was found that was common to both conditions. The QTL with the highest LOD (7.54) was qSN1.1. Like seed length, all qSN were contributed by HS584 (Supplemental Table S14; Fig. 1).

Co-location of QTL for different traits

Many of the crop agronomic characteristics are correlated with one another or could be dissected into individual components; it is thus natural to observe a particular QTL co-localizing for two or more different traits. In the present study, there were nine such QTL which co-localized for more than one trait and are herein referred to as "hot-spot QTL" (qHS) (Table 1; Fig. 1). The maximum number of qHS was detected on chromosome 1H (three), followed by two each on 3H and 7H. Of the qHS, qHS1.3 and qHS2.1 had the highest number of traits co-located (nine). The seven major hot-spot QTL (qHS1.1, qHS1.3, qHS2.1, qHS3.1, qHS3.2, qHS6.1 and qHS7.1) together harboured more than 80 % of the stable QTL detected in the present study.

Selection and phenotypic comparison of contrasting lines

Selection of contrasting lines was primarily carried out based on seven hot-spot QTL (Table 1). In addition to these, qYLD3.1 and qTGW3.1 were also considered during selection as they were two important HS584-derived QTL for yield and TGW, respectively. In general, the presence of HS584 segments at the hot-spot QTL significantly reduced the trait value for major Brenda-contributed traits like yield, TGW, starch amount per seed and seed breadth. Hence, the selected superior genotypes were characterized by few or no introgressions at these hot-spot QTL, together with the presence of beneficial HS584 QTL at qYLD3.1, qTGW3.1 and qTGW3.2. On the other hand, inferior

Table 1 Details of major hot-spot QTL

QTL[a]	Marker[b]	cM[c]	Col. QTL[d]	Col. traits[e]	Source[f]	Con.[g]	Str.[h]
qHS1.1			qDTI-Y1.1	DTI-Y	Brenda	Yes	NA
	GBS3171	60.1	qTGW1.1	TGW	Brenda	Yes	Yes
			qDTI-GW1.1	DTI-TGW	Brenda	Yes	NA
	Bmac90	78.5	qSL1.1	Seed length	HS584	Yes	No
			qSLB1.1	Seed L/B	HS584	Yes	No
qHS1.3			qYLD1.3	Yield	Brenda	Yes	Yes
			qDTI-Y1.2	DTI-Y	Brenda	Yes	NA
			qTGW1.3	TGW	Brenda	Yes	Yes
	GBS3269	98.8	qDTI-GW1.3	DTI-TGW	Brenda	Yes	NA
			qSLB1.2	Seed L/B	HS584	Yes	No
			qSL1.2	Seed length	HS584	Yes	No
	GBS3272	127.8	qSB1.1	Seed breadth	Brenda	Yes	Yes
			qSS1.1	Seed starch	Brenda	Yes	Yes
			qSN1.1	Seed nitrogen	HS584	Yes	No
qHS2.1			qYLD2.1	Yield	Brenda	Yes	No
			qDTI-Y2.1	DTI-Y	Brenda	Yes	NA
			qTGW2.1	TGW	Brenda	Yes	Yes
	GBS3273	67.5	qDTI-GW2.1	DTI-TGW	Brenda	Yes	NA
			qSLB2.1	Seed L/B	HS584	Yes	Yes
			qSL2.1	Seed length	HS584	Yes	Yes
	GBS3279	77.6	qSB2.1	Seed breadth	Brenda	Yes	Yes
			qSS2.1	Seed starch	Brenda	Yes	Yes
			qSN2.1	Seed nitrogen	HS584	Yes	No
qHS3.1			qSB3.1	Seed breadth	Brenda	Yes	Yes
			qSS3.1	Seed starch	Brenda	Yes	Yes
	GBS3145	41.9	qSN3.1	Seed nitrogen	HS584	Yes	No
			qSLB3.1	Seed L/B	HS584	Yes	Yes
qHS3.2			qTGW3.2	TGW	HS584	Yes	No
	Bmag225	74.3	qSN3.2	Seed nitrogen	HS584	No	Yes
qHS6.1			qTGW6.1	TGW	Brenda	Yes	Yes
	GBMS222	71.1	qDTI-GW6.1	DTI-TGW	Brenda	Yes	NA
			qSLB6.1	Seed L/B	HS584	Yes	Yes
	Bmag613	79.8	qSB6.1	Seed breadth	Brenda	Yes	Yes
			qSS6.1	Seed starch	Brenda	Yes	Yes
			qSN6.1	Seed nitrogen	HS584	Yes	No
qHS7.1			qTGW7.1	TGW	Brenda	Yes	Yes

Table 1 continued

QTL[a]	Marker[b]	cM[c]	Col. QTL[d]	Col. traits[e]	Source[f]	Con.[g]	Str.[h]
	GBS3128	0	qSB7.1	Seed breadth	Brenda	Yes	Yes
			qSS7.1	Seed starch	Brenda	Yes	Yes
	GBS3159	5.4	qSLB7.1	Seed nitrogen	HS584	Yes	Yes

NA not applicable

[a] qHS refers to hot-spot QTL where many traits were co-located; the first number corresponds to the chromosome and the second number corresponds to the order of the hot-spot QTL detected in that particular chromosome

[b] Marker with significant QTL effect

[c] Position of the markers in centiMorgans (cM)

[d] Different co-locating QTL at a particular hot-spot QTL (refer to Fig. 2) with 'q' referring to QTL, followed by the trait name, chromosome number and the order of the QTL detected for a particular trait in that chromosome

[e] Different co-locating traits at a particular hot-spot QTL; trait abbreviations are given in Supplementary Table S2

[f] Refers to the allele from the parent which increases the trait value

[g] QTL was detected under control condition

[h] QTL was detected under stress condition

genotypes were characterised by the presence of HS584 introgressions at the major hot-spot QTL. Accordingly, DH21, 22, 23 and 24 were categorized as superior lines and DH5, 44, 56, 57 and 58 as inferior. Among the superior genotypes, DH22 was the only genotype which had all beneficial HS584 segments (QTLYLD3.1, QTLTGW3.1 and QTLTGW3.2). DH23 and 24 did not possess HS584 introgression at qYLD3.1, whereas DH21 did not have it at qTGW3.2 (Fig. 2). In addition, all superior genotypes had the introgression at qTGW3.1. Another notable feature among the superior genotypes was that DH23 had an HS584 introgression at a major Brenda hot-spot QTL, qHS6.1. Among the inferior lines, DH5 and 44 had HS584 introgressions at the major hot-spot QTL qHS1.1, qHS1.3 and qHS2.1. A schematic representation of the selected contrasting genotypes along with the QTL considered for their selection is shown in Fig. 2.

The average yield and TGW obtained from four different experiments carried out at Nordsaat during 2007 and 2008 indicated that inferior lines were characterized by lower yield and TGW than superior

Fig. 2 Schematic representation of selected contrasting genotypes. The selected DHs are grouped into superior and inferior genotypes based on major hot-spot QTL and the presence of positive HS584 segments (qYLD3.1 and qTGW3.1) mainly influencing yield and TGW. Superior genotypes are characterised by the presence of HS584 segments (qYLD3.1 and qTGW3.1) positively influencing yield and TGW, whereas inferior genotypes are characterised by the presence of HS584 segments negatively influencing yield and TGW. Introgression of HS584 segments positively influencing yield and TGW (also seed breadth, seed starch) are shaded in *grey*, whereas the negatively influencing HS584 segments for the above traits are marked with *hatching*

Fig. 3 Effect of HS584 introgressions for yield and TGW in the contrasting genotypes. **a** Yield (combined across four experiments from Nordsaat) under control and drought conditions for the selected contrasting genotypes. Yield from Nordsaat field experiment, where the whole plot was used for yield estimation, was normalized to other experiments (yield from 10 plants). Values are mean ± SE ($n = 4$). **b** TGW (combined across four experiments from Nordsaat) under control and drought conditions for the selected contrasting genotypes. Values are mean ± SE ($n = 4$). Based on HS584 introgression in the background of Brenda, DH21, 22, 23 and 24 have been categorized as superior lines and DH5, 44, 56, 57 and 58 as inferior. See Fig. 2 legend for the classification of contrasting genotypes into superior and inferior
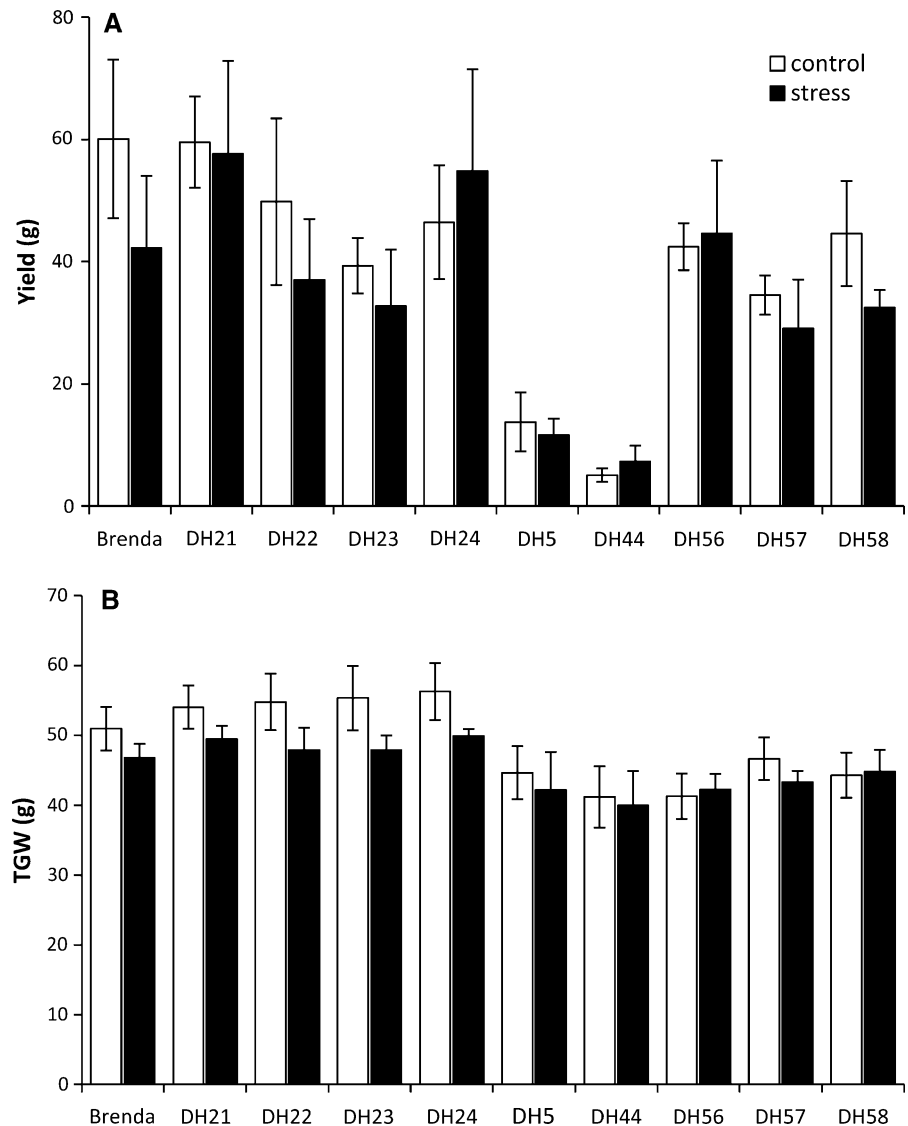
ones (Fig. 3a, b). Comparison of superior genotypes to Brenda indicated that yield was not very different between these two, especially under control conditions; however, there was a clear tendency of higher TGW in superior genotypes compared to Brenda under both control and drought conditions. The poor performance of DH5 and 44 among the inferior lines could be attributed to the fact that they had HS584 introgressions at all three major Brenda yield QTL (Fig. 2) while the others were devoid of introgression at qHS1.1. Similarly, starch amount per seed obtained from the IPK greenhouse experiment showed that

inferior lines were generally low in starch content and were more susceptible to drought than superior lines (Fig. 4a). Like TGW, seed starch content was generally higher in the superior genotypes than in Brenda under both conditions. There was no major difference between the contrasting groups in terms of seed nitrogen content under control conditions; however, drought stress significantly increased the seed nitrogen content of inferior lines over their control compared to superior genotypes (Fig. 4b). A significantly reduced C/N ratio of inferior genotypes under drought over their control compared to superior ones indicates that
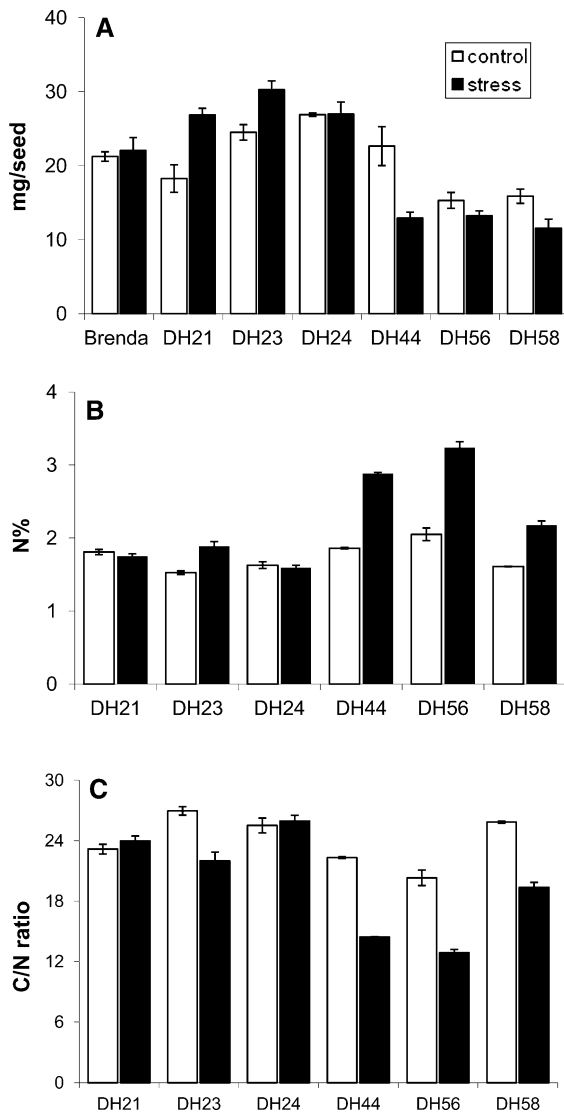
Fig. 4 Effect of HS584 introgression for seed quality parameters in the contrasting genotypes. **a** Seed starch amount per seed was generally high in the superior genotypes under both control and drought conditions compared to Brenda and inferior genotypes. **b** Seed N% was significantly increased under drought over control in the inferior genotypes compared to superior ones. **c** C/N ratio was significantly increased under drought over control in the inferior compared to superior genotypes. All values are mean ± SD ($n = 4$) and values were not determined in Brenda for seed N% and C/N ratio. Based on HS584 introgression in the background of Brenda, DH21, 23 and 24 have been categorized as superior lines and DH44, 56 and 58 as inferior

starch and nitrogen metabolism of the contrasting groups were differentially affected by drought (Fig. 4c).

Discussion

Phenotypic analysis for terminal drought

Wild barley represents a repertoire of favourable alleles for a variety of traits including terminal drought tolerance (Ellis et al. 2000; Talame et al. 2004; Pillen et al. 2004; Inostroza et al. 2009; Lakew et al. 2011, 2013; Schnaithmann and Pillen 2013). The present study was undertaken to identify the exotic genomic regions influencing yield and seed quality traits under terminal drought using a BC3-DH population developed by Li et al. (2006) between a German two-rowed cultivar, Brenda, as the recurrent parent and the wild accession, HS584, as the donor parent.

In the present study, terminal drought under field condition was mimicked by spraying crop canopy with potassium iodide (KI), 0.4 % at 8 DAF. Application of crop canopies with chemical desiccants such as KI has been used in screening for terminal drought tolerance in a variety of crops to mimic drought-mediated impairment in photosynthesis efficiency and to test its impact on TGW. The reduction in TGW obtained with chemical spray was generally well correlated with the reduction observed under terminal drought (Nicholas and Turner 1992; Blum 1998; Sawhney and Singh 2002). However, in our study, TGW obtained with KI treatment was poorly correlated with TGW obtained from the imposed drought experiment in the greenhouse. A similar finding was reported by Gavuzzi et al. (1997), who found that chemical desiccant treatment was less reproducible than other laboratory-based tests and suggested that in wheat the extent of damage caused by chemical desiccant was greatly influenced by growing conditions. A possible reason for the above finding in the present study could be due to the spraying of KI to the whole plant including spikes, which results in poor sink (Nicholas and Turner 1992). This could also be the reason for a poor correlation observed between yield and TGW in the field but not in the greenhouse under drought conditions.

Phenotypic evaluation of the mapping population under different environmental conditions showed that the two parents differed significantly in various traits scored (Supplemental Tables S3 and S4), and transgressive segregation was evident in the progenies for most of the traits, indicating the presence of both positive and negative alleles of a trait in both parents.

A positive correlation of yield and TGW under all greenhouse experiments together with a high correlation in TGW compared to yield between different environments (Table 1) reinforces the suitability of TGW as a better parameter to screen for tolerance to terminal drought. In addition, a strong positive correlation of TGW with starch amount per seed under both control and drought conditions (Supplemental Table S6) also indicates that in addition to screening for yield, TGW could also be a used as potential parameter to screen for quality parameters, such as seed starch in barley, as grain weight is mostly constituted by starch. Significant positive correlations in yield, TGW, DTI-Y and DTI-GW between the treatments in different experiments (Table 1) indicate that it is the genetic potential of the crop that determines tolerance under drought. Consistent with other studies in cereals, yield and TGW were negatively correlated to seed N%, which has been strongly attributed to source limitation in N supply and to the dilution effect of N by carbon reserves (Munier-Jolain and Salon 2005; Acreche and Slafer 2009).

QTL analysis

QTL analysis of different traits showed that a significant number of QTL (42 %) was contributed by the wild accession, HS584, considering both control and drought conditions together. Similarly, the wild parent was found to contribute 34 and 52 % of the favourable QTL detected in two previous studies by Pillen et al. (2003) and Talame et al. (2004), respectively, using AB-QTL analysis.

One of the major hindrances to QTL analysis is the instability of QTL as a result of large environmental effects. In this regard, QTL obtained in the present study could be relatively more stable as the plants were grown in diverse environments during different years and only common QTL that appeared in at least two different environments were considered for analysis. Of the stable QTL, 46 % were contributed by the wild parent; a notable feature of these QTL was the lower stability compared to Brenda, especially for yield and TGW characters (Supplemental Table S11 and S12). A probable reason for the above could be the selective breeding of the cultivated parent for its yield stability under diverse environments.

QTL detected in a particular population relate to the genetic background of the population studied.

Comparisons were made with similar studies that have used different populations, either to reinforce the importance of common QTL detected in these studies or to highlight a novel QTL controlling a particular trait in a specific population. In this context, QTL detected in the present study mainly for yield and TGW were compared to previous studies of AB-QTL analysis in barley. Comparison with previous studies has been made whenever possible using the consensus markers present between the studies or by comparing the markers present in the different studies to a barley consensus linkage map published by Varshney et al. (2007) using 765 SSR markers. QTL analysis of the seed morphological traits in barley was rarely addressed in the past and we could find only two studies to date addressing these traits: a recently published report by Schnaithmann and Pillen (2013) and another for seed length (Backes et al. 1995).

Yield and its drought tolerance index

A number of QTL studies have been performed in barley for yield and its contributing characteristics using wild barley as one of the parents (Pillen et al. 2003; Talame et al. 2004; von Korff et al. 2006; Lakew et al. 2013; Schnaithmann and Pillen 2013). The QTL, qYLD1.1, corresponding to the marker Bmac0154 was found to match a QTL reported by Talame et al. (2004); in both these studies, this particular QTL was derived from the cultivated parent. qYLD2.1 could also be mapped to similar locations, as reported by Talame et al. (2004) and Lakew et al. (2013); however, its precise location could not be verified due to the lack of common markers between the studies. However, the yield QTL (qYLD2.1) corresponding to the marker EBmac0684 was found to match a TGW QTL and a QTL for grain carbon isotope discrimination (Δ) detected by Teulat et al. (2001, 2002), respectively, using the population developed from a cross between Tadmor and Er/Apm grown under Mediterranean conditions. Carbon isotope discrimination is a surrogate for water use efficiency and hence grain yield, especially under conditions of limited water availability (Farquhar and Richards 1984). Grain Δ has been found to be the most appropriate parameter for grain yield under Mediterranean conditions (Voltas et al. 1998; Merah et al. 2001). The co-location of yield (present study), TGW and grain Δ at the marker EBmac0684 indicates that this could be a region

influencing grain yield under terminal drought. Another interesting QTL derived from a wild genotype is qYLD3.1, as this was not reported in earlier AB-QTL studies addressing yield under Mediterranean environments. QTL for drought tolerance index (qDTI-Y) were co-localized with QTL for yield, except for the exotic qDTI-Y1.3, indicating that it is the genetic potential of barley for yield that determines the yield under terminal drought. This is consistent with the idea that a drought-tolerant variety in a crop species would also be the one with higher yield potential under optimal conditions (Blum 2005).

Thousand-grain weight and its drought tolerance index

Thousand-grain weight or seed weight in barley is an important trait determining yield and quality of the product and has been one of the main targets of selective breeding during domestication in a number of cereals (Doganlar et al. 2000). A significant correlation in TGW between different environments with a higher magnitude than yield (Table S5) is consistent with the fact that TGW is a more heritable trait than yield.

In the present study, a total of eleven QTL were detected for TGW, and at two locations (qTGW3.1 and qTGW3.2) the increase in TGW under control conditions was contributed by HS584. Li et al. (2006), using the same population, found a total of 15 QTL for TGW which were distributed to all seven linkage groups, and at eight loci the increased TGW was contributed by HS584. Two QTL, qTGW1.3 (GBS3269) and qTGW6.1 (GBMS222) derived from Brenda in the present study, could be matched to the ones detected earlier by Li et al. (2006), indicating the stability of these QTL over different environmental conditions. A significant reduction in the number of QTL detected for yield and TGW in the present study compared to those of Li et al. (2006) could be due to the lower number of progeny lines analyzed in the present study [67 in the present study and 120 by Li et al. (2006)] or due to the strict criteria of QTL assignment (LOD ≥3 with appearance in a minimum of two environments), although the effect of environment cannot be ignored.

Two QTL, qTGW1.3 and qTGW2.1, corresponding to the markers Bmag0382 and Ebmac0684, respectively, were found to match the ones detected by

Lakew et al. (2013); however, qTGW1.3 represented a yield QTL in the later study. The qTGW2.1 was also found to correspond to one of the QTL detected by Schmalenbach et al. (2009) for TGW, although in the later study it was contributed by the wild accession. Similarly, qTGW1.1 and 3.1 were also found to match to similar locations to Lakew et al. (2013), although their precise location could not be verified due to a lack of common markers between the studies. A QTL near the marker Bmag225 (qTGW3.2) detected in the present study was also reported by Talame et al. (2004) and Teulat et al. (2002), indicating the influence of a particular gene or genes controlling TGW in different genetic backgrounds. Similarly, qTGW6.1 could be traced to similar locations as found by Talame et al. (2004) and Teulat et al. (2002) after comparing the markers between the studies to the consensus linkage map developed by Varshney et al.(2007). Like the QTL for qDTI-Y, all qDTI-TGW were co-localized with QTL for TGW.

The presence of both treatment-specific and constitutive QTL for yield and TGW (Supplemental Tables S11 and S12) indicates that crop yield under drought is determined by the concerted action of both constitutive and drought-specific expression of genes. The large number of QTL detected in Brenda for yield and TGW compared to HS584 could probably be due to the selection pressure applied on the cultivated parent for yield during domestication.

QTL for seed morphology

Grain morphological traits such as length, breadth, shape and density are important sub-components contributing to seed weight as well as grain quality in cereals (Kovach et al. 2007). A number of studies have addressed these parameters in rice and wheat (Gegas et al. 2010; Li et al. 2011). Although grain size and shape parameters are found to be associated with flour quality such as protein content and hydrolytic enzymes, which in turn determine the end quality of the seed, this has been largely overlooked in barley. Schnaithmann and Pillen (2013) recently reported an AB-QTL analysis where seed morphological characters such as breadth, length and area were analyzed using an IL population developed between the cultivated parent Scarlett and the wild accession ISR42-8.

A QTL study which included seed length in barley was reported by Backes et al. (1995) using a DH

population developed between Igri and Danilo using restriction fragment length polymorphism markers where two QTL for seed length were detected on chromosomes 4H and 7H. In the present study, a total of four QTL were detected for seed length (Supplemental Table S13) which were all derived from HS584, the parent superior in this trait, but none of them was mapped to chromosomes 4H or 7H. On the other hand, Schnaithmann and Pillen (2013) reported three QTL for seed length, of which two were located on chromosome 1H and the other on chromosome 7H. Although two QTL were also identified in our study on chromosome 1H, it could not be verified with the above study. Hence, the additional QTL identified on chromosomes 2H and 5H in this study are novel QTL for seed length in barley. Another notable feature of this trait was that none of the DHs had greater seed length than HS584 (Supplemental Table S3 and Supplemental Figure S3A). The above observation, together with the identification of all seed length QTL from HS584, indicates that Brenda did not possess any positive alleles for seed length. Detection of most of the seed length QTL only under control conditions, except qSL2.1 that was expressed in both conditions, indicates stability and a constitutive nature of qSL2.1 in influencing seed length in this population.

With regard to seed breadth, to the best of our knowledge there has been only one study reported in barley (Schnaithmann and Pillen 2013), although quite a number of studies have been carried out in other crops such as wheat, rice and tomato which also resulted in map-based cloning of some of the QTL identified (Frary et al. 2000; Fan et al. 2006). A total of six QTL were detected for seed breadth which were all derived from Brenda, the parent having a higher trait value. Similarly, Schnaithmann and Pillen (2013) also reported six QTL for seed breadth which were distributed to all linkage groups except 1H and 5H. However, in our study a QTL could be located to chromosome 1H, although in both studies QTL for seed breadth could not be located to chromosome 5H. At two locations, QTL for seed breadth (qSB1.1 and qSB2.1) and seed length (qSL1.2 and qSL2.1) were co-localized. Although it cannot be resolved from this study whether such co-location is due to linkage drag or pleiotropic effects, similar studies in rice found that QTL for seed weight (GS3 and GW2) had pleiotropic effects on both grain width and length (Fan et al. 2006; Takano-Kai et al. 2009). However, this is in contrast to

Schnaithmann and Pillen (2013) where no QTL for seed length and breadth were co-localized, indicating these two traits could be altered independently.

The trait seed L/B, which indicates seed shape, had a total of ten QTL, which were all derived from HS584 except for qSLB 5.1 and qSLB7.2. Although in most cases qSLB were co-localized with either seed breadth or length QTL, there were many independent QTL located on chromosome 5H (qSLB5.1, qSLB5.2 and qSLB5.3) and chromosome 7H (qSLB7.2), hinting at the genes that may specifically influence seed shape in barley. In tomato, it was found that seed size and shape are largely controlled by independent genes (Frary et al. 2000; Van der Knaap and Tanksley 2001). However, in a recent study for grain size and shape variation in wheat using six different mapping populations, Gegas et al. (2010) found that many of the QTL that affected grain size also influenced grain shape. Another interesting observation was that L/B was negatively correlated with traits mainly contributed by Brenda, like TGW, seed breadth and starch amount per seed, and positively correlated with HS584-contributed traits, like seed nitrogen content and seed length (Supplemental Table S6). Given the nature and direction of the correlation between the traits, it can be assumed that the major QTL where these traits are co-localized are probably due to pleiotropic effects of the underlying genes; however, the possibility of linkage drag can only be excluded if the segregating population at this region is further developed.

Seed quality traits

Seed starch and N content are important parameters determining the end use of barley for malting. Generally a low protein concentration is desirable for malting as high protein interferes with starch degradation during malting. There is a strong negative correlation between yield and protein content in cereals (Briggs 1978). For seed nitrogen, all QTL detected under both conditions were obtained from the parent HS584, the parent that had the higher value for this trait. Two QTL, qSN2.1 and qSN7.1, were found to match the QTL contributed by the wild parent reported by Li et al. (2005) using a BC3-DH population developed between Brenda and *H. vulgare* ssp. *spontaneum* accession, HS213.

Similarly, qSN2.1 corresponding to the marker EBmac0684 was found to match the QTL for grain

protein content reported by von Korff et al. (2008) using AB-QTL analysis of a BC2-DH population developed between the spring barley cultivar Scarlett and the wild barley accession ISR42-8. Other QTL present on chromosomes 1H and 6H could not be matched to the above study because of a lack of common markers. Similarly, qSN6.1 was found to be located in a region for a major barley grain protein content locus as reported by See et al. (2002). Interestingly, all QTL detected for grain N% were either specific to control or drought conditions, indicating that the corresponding genes are not constitutively expressed. The QTL identified for enhanced seed nitrogen could be explored for specialized breeding programs of feed barley rather than for malt barley.

For starch amount per seed, a total of five and three QTL were detected under control and drought conditions, respectively, and were all derived from Brenda. Interestingly, all these QTL were co-localized with QTL for seed N% except for qSS7.1 which was control-specific. Co-localization of seed starch and N% and a strong negative correlation observed between these two traits (Supplemental Table S6) indicates that the universal negative correlation observed between yield and grain protein content in cereals is probably the result of pleiotropic effect of genes controlling these traits. For example, a grain protein concentration (GPC) locus identified in wheat and barley (Uauy et al. 2006; Jukanti and Fischer 2008) was found to increase grain protein content through early induction of leaf senescence, resulting in better remobilisation of leaf nitrogen to developing grains. Since leaf nitrogen is also essential for maintaining the photosynthetic capacity, it is speculated that a functional GPC locus might lead to reduced carbon assimilation resulting in negative correlation between grain protein content and yield (Jukanti and Fischer 2008). In barley and other cereals, grain N% and yield are negatively correlated (Acreche and Slafer 2009); as a result, the modern varieties with improved yield are often associated with reduced N% or grain protein content (Calderini et al. 1999). Using altered source–sink relations in wheat grown under Mediterranean conditions, Acreche and Slafer (2009) found that the lower N% of the modern-day wheat varieties is a result of increased grain number and that the negative correlation between yield and grain protein content could be broken if source strength is enhanced.

The detection of qSS7.1, which did not co-localize with seed nitrogen content, indicates that the relationship between yield or TGW and grain N% can also be altered if the genes underlying such QTL for enhanced starch content are identified and specifically altered. This is evident in a recent study by Weichert et al. (2010), who reported that increasing the sink capacity of the wheat grains through overexpression of a barley (*Hordeum vulgare*) sucrose transporter *HvSUT1* (SUT) under the control of an endosperm-specific Hordein B1 promoter (HO) has resulted in increased grain protein content in addition to seed weight. Similarly, seed-specific expression of a bacterial PEP carboxylase in *Vicia narbonensis* was found to increase seed protein content together with seed weight (Rolletschek et al. 2004). From the above discussion, it is clear that yield and protein relation in cereals could be altered by altering either source or sink strength. However, a more negative correlation observed between yield and grain protein content under terminal drought compared to optimal conditions in cereals like barley is attributed to the differential accumulation of starch and protein during grain filling in cereals (Morgan and Riggs 1981; Brooks et al. 1982).

Co-localization of traits

Since many of the agronomic characteristics are correlated, the phenomenon of different traits co-localizing to a QTL is observed in a variety of crops, including barley and wheat (Zhu et al. 1999; Quarrie et al. 2005). Such co-localization can be due either to tight linkage of genes underlying a particular QTL with nearby genes or to the pleiotropic effect of the gene/genes underlying a QTL. In tomato (*Solanum pennellii*), fine mapping of a QTL for fruit mass has resolved the QTL into three closely linked loci (Eshed and Zamir 1995). On the other hand, grain weight and size QTL associated with the *sdw1* locus (semi-dwarfing locus) in barley could not so far be separated indicating the pleiotropic effect of the *sdw1* locus on grain size (Barua et al. 1993). A similar observation is the association of *Vrs1*, determining row type in barley and located on chromosome 2H, with grain size in barley, with two-rowed genotypes having heavier grains (Karakousis et al. 2003). However, it is difficult to distinguish the effect of pleiotropy from a linked QTL in an initial mapping population due to restriction in population size or recombination events between

the linked loci. Hence, further fine mapping is needed to resolve the nature of such QTL detected in the present study.

Candidate genes underlying the important QTL

Some of the possible candidate genes underlying some of the QTL detected in the present study could be identified, because the linkage map developed by Worch et al. (2011) for this population also involved gene-based SNP markers for drought-regulated gene sets in addition to genomic SSR markers. We mapped drought-responsive MYB transcription factors to the regions on chromosomes 1H (GBS3286) and 3H (GBS3145). Several other transcriptional regulators were localized on chromosome 2H (GBS3215, GBS3217, GBS3143, GBS3149, GBS3243 and GBS3224). The qYLD3.1 which harbours the glutamate decarboxylase gene (GBS3192) was detected under control conditions. Among the QTL for biochemical traits, qSS2.1 is interesting as it contains two starch branching enzymes (GBS3257 and GBS3274) and a sucrose synthase gene (GBS3273). The qSN1.1, which also co-locates with many other traits, contains at least four amino acid metabolism genes (GBS3193, GBS3249, GBS3250 and GBS3251).

Among the candidate genes, glutamate decarboxylase (GAD) appears interesting because it is the first enzyme of the GABA (gamma-aminobutyric acid) shunt pathway which is involved in the unidirectional conversion of glutamate to GABA by decarboxylation. GABA is a four-C non-protein amino acid that has a primary role in central C–N metabolism which is particularly relevant under stress (Bown and Shelp 1997; Bouche and Fromm 2004). In a recent study where the deregulated GAD was overexpressed in *Arabidopsis* seeds under seed maturation-specific phaseolin promoter, the transgenic plants were characterized by an increased C–N metabolism resulting in higher C/N ratio compared to the wild type (Fait et al. 2008).

Other interesting possible candidate genes are starch branching enzyme and sucrose synthase that co-localize with qSS2.1. Starch branching enzymes introduce α-1,6-glycosidic bonds into α-polyglucans and hence determine the amylopectin structure. In cereal species such as barley and wheat, three classes of starch branching enzymes (SBE I, SBE IIa and SBE IIb) have been reported and they were the primary targets for altering the seed quality by altering the amylose to amylopectin ratio (Boyer and Preiss 1981;

Wei et al. 2009). Antisense repression of SBE IIa and IIb in barley has resulted in a significant reduction in TGW of the transgenic line compared to wild type (Regina et al. 2010). Sucrose synthase, which catalyses the conversion of sucrose to fructose and UDP-glucose, is considered to be the marker enzyme of sink strength in several crops including cereals (Sun et al. 1992; Wang et al. 1993; Kato 1995; Jiang et al. 2012). Its activity was found to be the major determinant of seed-filling duration in barley and wheat under both optimal and drought conditions (Chevalier and Lingle 1983; MacLeod and Duffus 1988). Whilst the activity and expression of SBE genes in rice were generally suppressed under both drought and heat stress (Yamakawa et al. 2007; Peleg et al. 2011), sucrose synthase activity was suggested not to be a limiting factor under drought in a number of studies (Ober et al. 1991; Dorion et al. 1996; Sheoran and Saini 1996). The control-specific expression of qSS2.1 indicates that genes underlying this QTL are negatively affected by drought and further fine mapping is needed to reveal the genes responsible in this region.

Selected contrasting lines and the importance of C/N ratio

Selected contrasting genotypes revealed that yield reduction in the inferior genotypes was primarily due to the integration of *H. vulgare* ssp. *spontaneum* segments at the major Brenda QTL influencing yield and TGW. A significantly lower yield and TGW in DHs 5 and 44 compared with other inferior lines indicated that qHS1.1 is an important region determining both yield and TGW. It is generally believed that N accumulation is less sensitive to drought than starch accumulation, resulting in higher N accumulation relative to starch under drought stress (Morgan and Riggs 1981; Brooks et al. 1982; Triboi and Triboi-Blondel 2001). This was also evident in the selected inferior lines where N% was significantly increased under stress over control compared to superior lines. This resulted in a significantly reduced C/N ratio in the inferior lines than the superior lines under drought. Although seed N accumulation is also influenced by a number of environmental factors other than drought, the results from our study indicate that the C/N ratio could be a potential parameter for screening drought tolerance, not just for seed quality but also for yield performance under terminal drought.

Conclusion

AB-QTL analysis of 67 BC3-DH lines of Brenda × HS584 revealed that wild barley is a potential source of favourable alleles for both yield and seed quality traits under terminal drought stress. Although, in general, introgression of the exotic segments into the cultivated background caused poor yield performance of the resulting DHs, identification of drought-specific yield QTL (qYLD3.1) and control-specific TGW QTL (qTGW3.1 and qTGW3.2) from HS584 illustrates the potential of wild barley for improving the drought tolerance of cultivated barley. In addition, identification of novel QTL for seed morphological traits such as seed breadth, length and shape, which had been addressed least in the past, could be an important asset for studying these traits in the future. Most of the traits for seed morphology were co-located; the presence of few independent QTL for seed shape indicates that seed shape in barley can be altered independently of either seed length or seed breadth. Similarly, identification of some of the independent QTL detected for starch amount per seed that did not co-locate with seed nitrogen content indicates that the general negative correlation observed between these two traits can be altered if candidate genes underlying these specific QTL are detected through fine mapping.

Co-location of the drought tolerance indices of yield and TGW with their corresponding traits under control conditions indicates that breeding for terminal drought tolerance for yield in barley could primarily be targeted towards identification of a genotype with higher yield potential under optimal conditions. In addition, the candidate genes discussed could be interesting targets for further elucidation of the mechanisms of terminal drought tolerance in barley, in particular to enhance starch accumulation for improving the grain quality for malting.

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