

Genetic relationships between preharvest sprouting and dormancy in barley

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Abstract Preharvest sprouting (PHS) and dormancy (DOR) can be problems in barley production and end use quality, especially for barley used for seed and malting. Three crosses previously analyzed for DOR inheritance, were reanalyzed for PHS and DOR inheritance using artificial rain to calculate sprout score (SSc) and measure alpha-amylase activity (AA). Germination percentage of untreated grain for DOR was also measured. The crosses are ‘Step-toe’/‘Morex’ (previously published), ‘Harrington’/TR306, and ‘Triumph’/Morex. Among the three crosses, DOR QTLs were located to six and PHS QTLs to five chromosomes, respectively. Chromosome 6H was never implicated. Previously identified DOR QTLs were confirmed in each cross, and most PHS QTLs coincided with DOR QTLs, but not all. Unique PHS QTLs were identified on chromosomes 1H (AA), 2H (SSc, AA), 3H (SSc, AA), and 7H (SSc, AA) and unique DOR QTLs on 1H, 2H, and 7H. Results indicate that PHS susceptibility and DOR are not always represented by opposite alleles at a locus. Some QTL regions for a given trait are conserved

across crosses and some are not. Several QTLs are suitable for marker-assisted selection to balance PHS and DOR in breeding new cultivars.

Keywords *Hordeum vulgare* · Preharvest sprouting · Dormancy · QTL · Sprout score · Alpha-amylase · Germination percentage

Introduction

Sound seed is the basis for stand establishment in commercial production, as well as end use quality in cereal crops. Germination is the principal component of sound seed. Two somewhat opposite yet related phenomena relate to germination: dormancy and preharvest sprouting. These two extreme phenomena must be balanced (not too high and not too low) in cereal grain production and utilization. This is especially true for grain used for malting.

Barley (*Hordeum vulgare* L.) seed used for malting and grain production requires rapid, uniform and complete germination. High or excessive dormancy may cause non-uniform germination, poor stand establishment, and low malt extract yield in commercial production and in the malt house. It also increases cost and potential damage from storage (Carn 1980). On the other hand, lack of or low dormancy may cause preharvest sprouting in the field. Preharvest sprouting, known as germination of seed on the mother plant under rainy conditions before harvest (Li et al. 2003), also causes

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adverse consequences in barley. It triggers the synthesis of endosperm degrading enzymes (Bewley and Black 1994), decreases in grain weight and rapid loss of seed viability (Li et al. 2003). Thus it is critical to develop barley cultivars that are resistant to sprouting between physiological maturity and completion of harvest, but not excessively dormant to ensure rapid and consistent germination after harvest for stand establishment and malting.

Much research has been done to identify and verify dormancy quantitative trait loci (QTLs) for preharvest sprouting resistance in barley, as dormancy has been viewed as the opposite trait to preharvest sprouting or defined as sprouting resistance (Barr et al. 2003). Dormancy QTLs have been detected in several barley crosses: Steptoe/Morex (Ullrich et al. 1993; Han et al. 1996, 1999; Gao et al. 2003), Harrington/TR306 (Takeda 1996; Ullrich et al. 2002), Chebec/Harrington (Li et al. 2003), Triumph/Morex (Prada et al. 2004), Mona/Wadi Qilt 23-39 (Zhang et al. 2005), and Stirling/Harrington (Bonnardeaux et al. 2008). Major and minor effect QTLs have been mapped throughout the seven barley chromosomes over the different crosses and across many environments. In the Steptoe/Morex cross, of a total of more than 20, four consistent QTLs were detected on chromosomes 4H, 5H, and 7H; two on chromosomes 1H and 5H in Harrington/TR306; one on chromosome 6H in the Blenheim/E224-3; three on chromosomes 2H and 5H in Triumph/Morex; two on chromosomes 2H and 5H in Chebec/Harrington; and six on chromosomes 1H, 2H, 3H, 4H, and 5H in Stirling/Harrington. In Mona/Wadi Qilt 23-39, an *H.v. vulgare/H.v. spontaneum* cross, eight QTLs were detected across all chromosomes except 6H (Zhang et al. 2005). These studies highlight the genetic complexity of dormancy and presumably preharvest sprouting in barley.

However, little research has been done on preharvest sprouting per se. Improvement of preharvest sprouting resistance solely by manipulating dormancy QTLs has been confounded due to multiple QTLs/genes, their interactions, and environmental effects. In addition, it is not clear whether dormancy genes control all preharvest sprouting or if there could be unique preharvest sprouting genes independent from dormancy genes. The existence of undetected independent preharvest sprouting QTLs may be possible. There have been preharvest sprouting studies on mechanisms associated with intact spikes, morphological features of ear and

awn for water-uptake/drying rate, and epicuticular waxes for water repellency which are not directly related to dormancy per se (Mares 1989; King and von Wettstein-Knowles 2000). Some of these features may not be controlled by dormancy QTLs. Also dormancy QTLs were mostly detected through germination tests (germination percentage) with threshed seeds on Petri-dishes. Whereas, preharvest sprouting QTLs may be detected with other more direct procedures such as: visual sprout scores measured by stimulating sprouting within intact spikes under artificially humidified or rain conditions and by measurement of alpha-amylase activity, a parameter widely used for on-going germination/sprouting process with little or no visible germination/sprouting present. Co-segregating QTLs for dormancy (via germination test) and preharvest sprouting (via alpha-amylase activity) on chromosomes 2H (minor effect) and 5H (major effect) were detected in the Chebec/Harrington cross (Li et al. 2003).

This paper follows a similar one reporting on genetic analysis of preharvest sprouting (PHS) and dormancy (DOR) in the six-row barley cross 'Steptoe' (S, feed type) by 'Morex' (M, malting type). In the S/M study, PHS and DOR QTLs were found on seven and eight regions of five and six chromosomes with effects ranging from 4 to 31% and 3 to 39%, respectively (Ullrich et al. 2008).

Previously, the two-row barley cross, 'Harrington' (H, malting type) by TR306 (TR, feed type) and the two-row 'Triumph' (T, malting type) by six-row Morex barley cross were genetically analyzed for dormancy (Takeda 1996; Ullrich et al. 2002; Prada et al. 2004) but not directly for PHS. Therefore, the objectives of this study were first to detect DOR and PHS QTLs in the H/TR and T/M crosses, second to compare the QTLs identified in the H/TR, and T/M crosses with those identified in S/M and other barley crosses, and third to determine the genetic relationship between DOR and PHS. This would provide better genetic understanding and basis to achieve a desirable balance between DOR and PHS in barley.

Materials and methods

Plant materials

The parent pairs, Harrington/TR306 and Triumph/Morex and their respective mapping populations of

150 and 106 double haploid lines (DHLs) with molecular marker maps (Kasha et al. 1995; Prada et al. 2004) were utilized in the study. All procedures were as described in Ullrich et al. (2008). The H/TR mapping population and parents were grown in the greenhouse (GH) in 2005 and field (F) at Spillman Agronomy Farm, Pullman, WA in 2006 in completely randomized designs. The T/M materials were grown in the GH in 2006 in completely randomized design. Standard barley growing conditions were used in the field in two 3 m long row plots. The greenhouse was set at 20/15°C for day and night with 16 h of photoperiod. Fifteen heads from each DHL, as well as the two parents, were randomly harvested from each row in each environment at two different times; physiological maturity (0d PM) when green color disappeared on spikes and stems and 14 days after PM (14d PM). All materials harvested were stored immediately at –20°C until analyses were conducted.

Preharvest sprouting simulation and measurement

To determine susceptibility to PHS, visual tests were performed to measure sprouting occurrence. A sprout score (SSc) procedure was modified from the artificial rain tests by DePauw and McCaig (1991). Two replications of five spikes from each plot with ~30 cm stems harvested in the greenhouse and field at 0d and 14d PM were tested in the mist chamber for 5 days. The mist was evenly spread from the top for 5 h on the first day then 30 min every 12 h thereafter. The SSc was visually determined on each dissected spike using a 0–5 scale: 0 = no visible roots or shoots, 1 = ≤3 roots, no shoots; 2 = ≤5 roots, ≤3 shoots; 3 = ≤8 roots, ≤5 shoots; 4 = >25% ≤50% roots and shoots; 5 = ≥50% roots and shoots. Only roots and shoots ≥2 mm long were counted. Mean SScs of the five replicated spikes scored were used for genetic analysis.

Analysis of grain alpha-amylase activity

Alpha-amylase activity (AA) analysis was used to measure degree of pre-germination with little or no visible sprouting when the sprouting score was ≤1. AA is considered a reliable indicator as it is synthesized de novo during the germination process. AA was determined in duplicate to achieve 10% agreement using the Megazyme Alpha-Amylase assay kit (<http://www.megazyme.com/booklets/KCERA.pdf>).

The mist-treated spikes from each harvest date and growing environment as described above with sprout score ≤1 were dried at 60°C for 48 h, hand-threshed, and milled. The enzyme activity was determined by a spectrophotometer set at 400 nm and expressed as units of enzyme per g of flour (Ug^{-1} flour).

Dormancy tests

To determine level of dormancy, standard germination tests were performed using two replicates of 50 seeds from 0d PM and 14d PM from each plot from each growing environment. Barley grain was germinated on moist filter paper in 100 mm Petri dishes in a dark incubator set at 20°C. Germinated seeds (root protrusion ≥2 mm) were counted for germination percentage (GP) after 7 days. Means of the two replications were used for genetic analysis.

QTL mapping procedure and genetic analyses

The mean phenotypic data (SSc, AA, and GP) for each harvest date from each growing environment were subjected to QTL analysis using MapManager QTX (Manly et al. 2001; www.mapmanager.org/mmqt.html). Likelihood Ratio Statistic (LRS) thresholds were established at 1 and 5% probability levels with 500 and 5,000 permutation tests, respectively. Regression, simple interval mapping (SIM, no background control for other QTLs) and composite interval mapping (CIM, background control for the most significant QTL) functions were utilized. Regression analysis involved whole genome scans, while SIM and CIM focused on individual chromosomes based on the putative regression results. Simple interval mapping identified the most significant QTL in a given trait analysis and subsequent QTLs were usually identified by CIM. Simple interval mapping and CIM QTL results significant at $P \leq 0.05$ are emphasized. QTLs indicated by regression analysis ($P \leq 0.05$) not confirmed with a significant LRS from interval mapping were treated as indicators of the potential for the existence of minor QTLs only, and were included in this paper only if they overlapped with SIM/CIM verified QTL intervals for other traits. Further, the H/TR and T/M DHL phenotypic data (GP and SSc) of a sample of QTLs displaying a range of QTL effects were averaged based on each genotype (allele) at marker loci with the highest LRS value

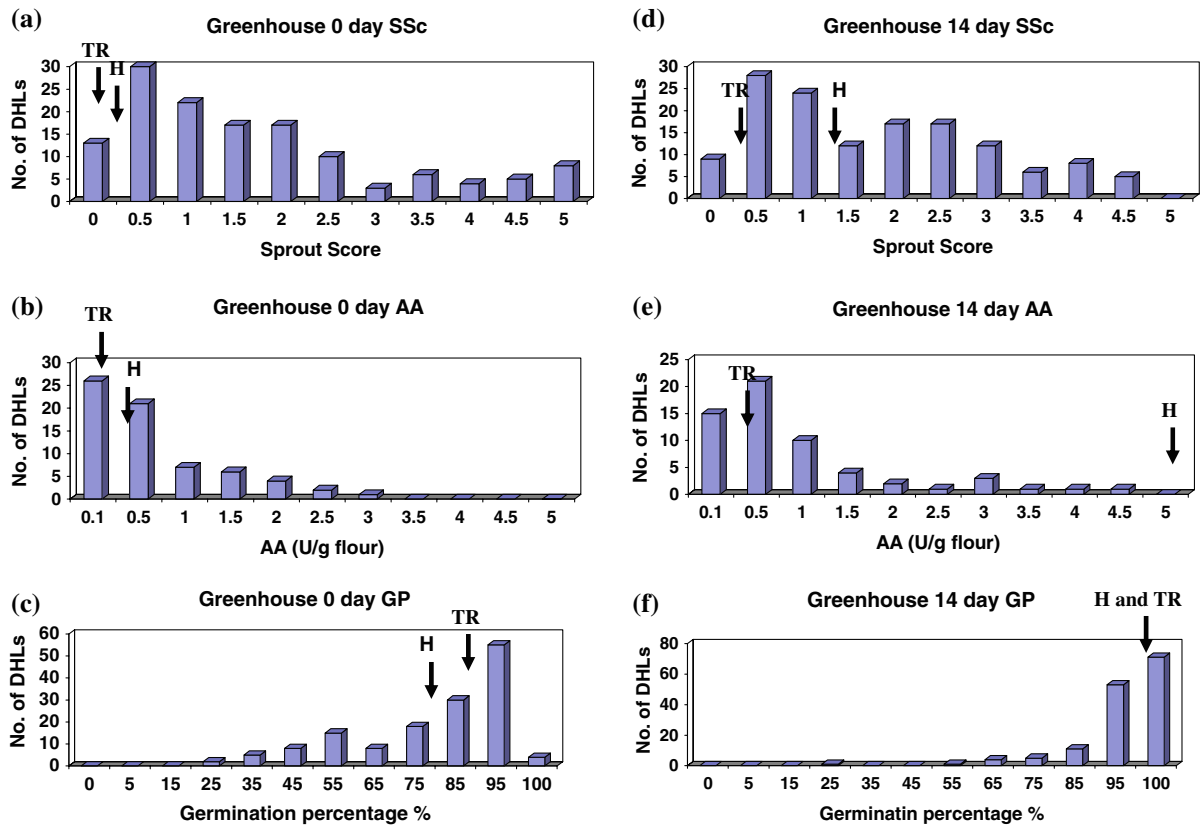


Fig. 1 Frequency distributions for the preharvest sprouting (sprout score, SSc; alpha-amylase activity, AA) and dormancy (germination percentage, GP) traits measured at 0 day (a–c) and

14 days (d–f) after physiological maturity from greenhouse environment in the Harrington (H)/TR306 (TR) doubled haploid mapping population and parents

(QTL plot peak). AA was excluded because of the limited number of lines analyzed due to the restricted criteria for measuring AA. The DHLs of each mapping population were divided according to their H or TR allele or T or M allele designation at a given marker for a given trait. The averages of H versus TR and T versus M type were separately calculated to detect mean differences affected by QTLs identified by SIM and/or CIM.

Results and discussion

Phenotypic data

Sprout scores for Harrington and TR306 were similar to each other at 0d PM regardless of environment (0.1 vs. 0.0 in GH; 2.2 vs. 2.3 in F), but were higher for Harrington at 14d PM (1.2 vs. 0.4 in GH; 1.6 vs. 0.4 in F), Table 1a, b. Even so, the SScs were quite low

indicating little expression of sprouting in either parent. However, the H/TR DHLs displayed a wide range of SScs from 0 to 5 or near five from each environment with 0 and 14d PM means of 1.4 and 1.8 (GH), 2.0 and 2.8 (F), respectively. Sprout scores for Triumph and Morex from the GH were 0.0 and 0.9 at 0d PM and 0.0 and 1.0 at 14d PM, respectively (Table 1c). The T/M DHL SScs ranged from 0 to 4.1 and 0 to 3.8 with means of 0.1 and 0.2 for 0 and 14d PM, respectively. The frequency distributions of SScs for both mapping populations were continuous and skewed toward low scores or PHS resistance (Figs. 1a, d and 2a, d).

Alpha-amylase activity for Harrington was only measurable ($0.284 \text{ U g}^{-1} \text{ flour}$) at 0d PM from the GH (Table 1a). For TR306, AA was 0.137 and 0.189 $\text{U g}^{-1} \text{ flour}$ for 0 and 14d PM, respectively, from the GH and above the threshold and $0.931 \text{ U g}^{-1} \text{ flour}$ for 0 and 14d PM, respectively, from the F (Table 1b). The AAs for the H/TR DHLs ranged from 0.012 to

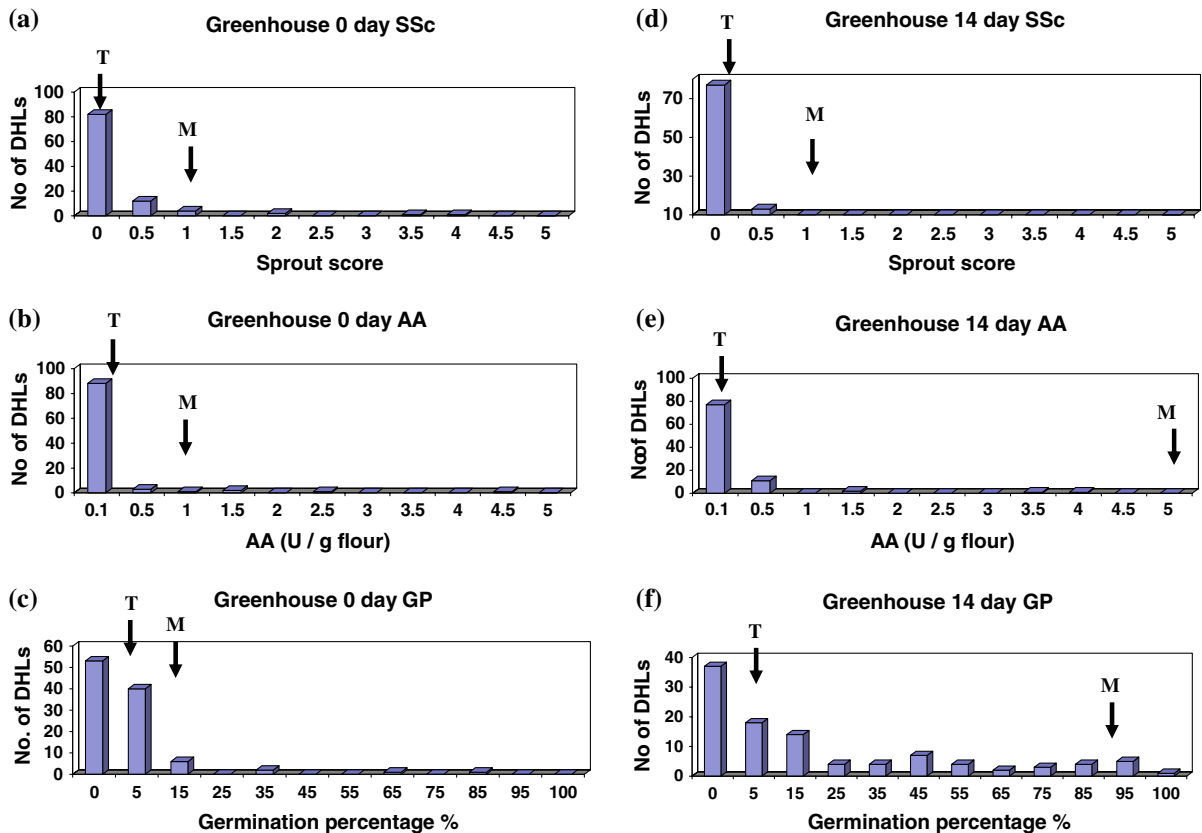


Fig. 2 Frequency distributions for the preharvest sprouting (sprout score, SSc; alpha-amylase activity, AA) and dormancy (germination percentage, GP) traits measured at 0 day (a–c) and

14 days (d–f) after physiological maturity from greenhouse environment in the Triumph (T)/Morex (M) doubled haploid mapping population and parents

3.087 Ug^{-1} flour (GH) and 0.021 to 4.407 Ug^{-1} flour (F) at 0d PM, and 0.038 to 4.508 Ug^{-1} flour (GH) and 0.106 to 3.656 Ug^{-1} flour (F) at 14d PM. For Triumph, AAs were 0.022 and 0.014 Ug^{-1} flour at 0 and 14d PM, respectively, from the GH, while AAs for Morex were 0.656 Ug^{-1} flour and above the threshold at 0 and 14d PM, respectively (Table 1c). The frequency distributions for AA s for both mapping populations were continuous and skewed toward low activities or PHS resistance (Figs. 1b, e and 2b, e).

Germination percentages measured from the GH and F were higher for TR306 (93 and 99%, respectively) than for Harrington (83 and 81%, respectively) at 0d PM, but were the same at both environments (99 or 100%) at 14d PM (Table 1a, b). The H/TR DHLs at 0d PM from the GH and F ranged from 20 to 100% and from 43 to 100%, respectively. At 14d PM the ranges were 28–100% and 89–100% from GH and F, respectively. The frequency distributions for GP were

continuous and skewed toward higher GPs or non-dormancy (Fig. 1c, f). Triumph (1%) and Morex (8%) germination percentages were both low at 0d PM, but were greatly different at 14d PM with Triumph remaining dormant at 3% GP, and Morex largely losing its dormancy with 95% GP (Table 1c). The T/M DHL GPs ranged from 0 to 92% at 0d PM and from 0 to 100% at 14d PM. The frequency distributions for GP at both after ripened periods were continuous and skewed toward low GPs or dormancy (Fig. 2c, f). In general, skewed frequency distributions are very common for dormancy and preharvest sprouting trait inheritance (QTL) studies (Ullrich et al. 1993, 2008; Kato et al. 2001; Li et al. 2003; Hori et al. 2007).

Comparing the phenotypic results of these two crosses and that of the previously published Steptoe/Morex cross (Ullrich et al. 2008), there are clear differences in phenotypic polymorphism between parents within crosses and parent pairs among crosses.

Table 1 Preharvest sprouting and germination (dormancy) means \pm standard errors of phenotypic data for Harrington (H), TR306 (TR), and 150 doubled haploid line (DHL) mapping population; (a) greenhouse and (b) field environments; and for Triumph (T), Morex (M), and 106 DHL mapping population; (c) greenhouse environment

Genotype	Physiological maturity (0d)			14d After-ripening (14d)		
	Sprout score	α -amylase U g^{-1} flour	Germination %	Sprout score	α -amylase U g^{-1} flour	Germination %
a Greenhouse H/TR						
Harrington	0.1 \pm 0.1	0.284	83 \pm 3	1.2 \pm 0.4	ND ^a	100 \pm 0
TR306	0 \pm 0	0.137	93 \pm 3	0.4 \pm 0.3	0.189	99 \pm 1
DHL Mean	1.4 \pm 0.4	0.496	78.6 \pm 3.7	1.8 \pm 0.4	0.717	95.0 \pm 1.6
DHL Minimum	0	0.012	20	0	0.038	28
DHL Maximum	5	3.087	100	4.7	4.508	100
DHL <i>n</i>		67			59	
b Field H/TR						
Harrington	2.2 \pm 0.7	ND ^a	81 \pm 9	1.6 \pm 0.4	ND ^a	100 \pm 0
TR306	2.3 \pm 0.6	ND ^a	99 \pm 1	0.4 \pm 0.2	0.931	100 \pm 0
DHL Mean	2.0 \pm 0.4	1.740	83.8 \pm 3.4	2.8 \pm 0.3	1.519	99.6 \pm 0.3
DHL Minimum	0	0.021	43	0	0.106	89
DHL Maximum	4.9	4.407	100	5	3.656	100
DHL <i>n</i>		22			7	
c Greenhouse T/M						
Triumph	0 \pm 0	0.022	1 \pm 1.0	0 \pm 0	0.014	3 \pm 0.7
Morex	0.9 \pm 1.1	0.656	8 \pm 7.9	1 \pm 1.1	ND ^a	95 \pm 1.0
DHL Mean	0.1 \pm 0.01	0.134	5.9 \pm 0.6	0.2 \pm 0.03	0.162	22.7 \pm 0.2
DHL minimum	0	0	0	0	0.002	0
DHL maximum	4.1	4.289	92	3.8	4.064	100
DHL <i>n</i>		96			92	

^a Not determined: Sprout score above the threshold for measuring α -amylase activity

Whereas, it has been assumed that Harrington has less capacity for dormancy or resistance to PHS, there was little difference between Harrington and TR306 in GPs, SScs, and AAs, except at 14d PM from the GH environment. There were few relative differences between Triumph and Morex for the three traits at 0d PM, but there were clear differences at 14d PM. On the other hand, Steptoe and Morex displayed great phenotypic polymorphism for the three traits. Interestingly, with the H/TR cross, the frequency distributions were skewed toward high GPs and low SScs. This relationship is not expected as lack of dormancy should promote PHS susceptibility. This may indicate that there can be partial independent genetic control. The T/M and S/M crosses produced expected consistencies in their frequency distributions; skewing toward low GP (dormancy) and low SSc and AA (PHS resistance). Transgressive segregation was apparent for most trait measurements, and when it

occurred, it was one-sided, since the parents tended to be clustered at one end of the trait range. With the S/M population, there was less evidence of transgressive segregation as the parents tended to be at opposite extremes of the trait range. Regardless of parental phenotypic polymorphism levels, there were broad and continuous DHL ranges, which are evidence for parental genotypic polymorphism and quantitative trait inheritance, both requisite for successful QTL detection.

QTL mapping

Regression analyses (Reg) detected nearly 50 QTLs ($P \leq 0.05$) in 14 chromosome intervals on five chromosomes associated with the three traits measured in the two crosses (data not shown). QTLs detected by SIM and/or CIM ($P \leq 0.05$) were considered real, while those by Reg only as putative or very minor. In

all, 25 QTLs were detected by interval mapping with individual QTLs explaining 6–40% and 6–54% of the phenotypic variation in H/TR and T/M, respectively. Similar high QTL detection numbers have been reported previously for DOR in S/M (Ullrich et al. 1993) and for DOR/PHS in a bread wheat cross (Kulwal et al. 2005). Overlapping QTLs for different traits were detected in four chromosome regions by interval mapping, which may indicate pleiotropy or clusters of closely linked genes (Li et al. 2003). Furthermore, some QTLs showed multiple peaks suggesting the possibility of more than one gene involved in a QTL chromosome interval.

Preharvest sprouting QTLs: sprout score and alpha-amylase activity

Five SIM/CIM-verified SSc QTLs were detected on chromosomes 2HS, 2HC (C refers to centromeric region), 3HL, 5HL and 7HC in the H/TR population (Table 2; Fig. 3a, b) and one QTL on chromosome 5HC in the T/M population (Table 3; Fig. 4a). The three chromosome 2HS, 2HC, and 7HC QTLs in H/TR had relatively minor effects explaining 6–7% (R^2) of the variation with TR306 contributing the additive alleles reducing SSc by 0.4 at each QTL. The two QTLs on chromosomes 3HL and 5HL in H/TR had relatively moderate effects ($R^2 = 12$ –17%) and again TR306 reduced SSc by 0.5 at each QTL. The single SIM/CIM-verified SSc QTL in T/M on chromosome 5HC had a major effect explaining 33% of the variation with Triumph contributing the additive alleles reducing SSc by 0.4. In the Steptoe/Morex cross, SSc QTLs were identified on chromosomes 2HS, 3HC, 4HS, 5HC, 5HL, and 7HC (Ullrich et al. 2008). It appears that SSc QTLs identified in the chromosome 5HC region of S/M and T/M may be the same, and the SSc QTLs in the chromosome 2HS, 5HL telomere, and 7HC regions of S/M and H/TR may be the same. These tentative conclusions are based on common markers in the QTL intervals on the barley bin map (<http://barleygenomics.wsu.edu/>).

Alpha-amylase activity QTLs in H/TR were identified on chromosomes 3HL, 5HL and 7HS, all with relatively minor effects of 6–8% (Table 2). TR306 contributed the alleles for PHS resistance (lower AA) at the chromosome 3HL and 5HL QTLs, while, Harrington contributed the PHS resistant allele at the chromosome 7HS QTL. From T/M, AA QTLs were

identified on chromosomes 1HS and 5HC (Table 3; Fig. 4b, c). Morex contributed the PHS resistant (low AA) allele at the relatively minor ($r^2 = 10\%$) QTL on chromosome 1HS, while Triumph contributed the PHS resistant allele at the major (R^2 up to 38%) chromosome 5HC QTL. The T/M AA QTL on chromosome 5HC also coincides with the 5HC SSc QTL in T/M and AA and SSc QTLs on 5HC in S/M (Ullrich et al. 2008). The chromosome 5HL AA QTL detected in H/TR coincides with the SSc QTL in the same 5HL region in H/TR and the AA QTL in the Chebec/Harrington cross (Li et al. 2003), as well as, AA and SSc QTLs in the same region in the S/M cross (Ullrich et al. 2008).

Dormancy QTLs: germination percentage

Five DOR QTLs were detected on chromosomes 1HL, 2HS, 5HL, and 7HC in the H/TR population (Table 2; Fig. 3c, d). The chromosome 1HL and 5HL QTLs in the current study confirmed those previously identified in H/TR (Takeda 1996; Ullrich et al. 2002), Chebec/Harrington (Li et al. 2003), and Stirling/Harrington (Bonnardeaux et al. 2008). The chromosome 5HL telomere DOR QTL also coincides with a DOR QTL region identified in the S/M cross (Ullrich et al. 1993, 2008; Han et al. 1996; Gao et al. 2003), and was named SD2 from the initial dormancy QTL work with the S/M cross (Han et al. 1996). Two newly identified DOR QTLs in H/TR were located to chromosomes 2HS and 7HC (=SD3 in S/M). Each of the four QTLs on chromosomes, 1HL, 2HS and 7HC explained 7–10% of GP variation. Harrington contributed the dormant alleles for the two chromosome 1HL QTLs with GP reductions of 3.1–6.3%, while TR306 contributed the dormant alleles for the chromosome 2HS and 7HC QTLs with GP reductions of 3.6 and 2.7%, respectively. The QTL region on chromosome 5HL had the greatest effect on GP explaining up to 40% of variation with up to 12.3% GP reduction due to the TR306 allele. From the T/M cross, four DOR QTL regions were mapped to chromosomes 3HL, 5HC, 7HC (=SD3 in S/M), and 7HL (Table 3; Fig. 4d). The non-dormant parent Morex contributed the dormant alleles of the moderate effect ($R^2 = 13\%$) QTL on chromosome 3HL and the two minor effect ($R^2 = 6$ –7%) chromosome 7H QTLs. Triumph, the dormant parent, contributed the dormant allele of the major effect (R^2 up to 54%) DOR QTL on chromosome 5HC. This

Table 2 QTL regions affecting preharvest sprouting and dormancy in the Harrington/TR306 cross

Chromosome	Flanking markers	Trait ^a	%Variation explained	LRS significance	Additive effect ^b
1HL	iPgd2- TubA2 (3.9 cM)	GP 0d G	10	16: **SIM/CIM	6.3 (H)
		MWG2028- Aga7 (20.1 cM)	SSc 0d G	3	4: *Reg
		GP 0d G	10	15: **SIM/CIM	6.1 (H)
		GP 14d G	8	12: **SIM/CIM	3.1 (H)
2HS	ABG058- MWG878A (6.8 cM)	GP 14d F	4	5: *Reg	0.3 (H)
		SSc 14d G	7	10: *SIM/CIM	0.4 (TR)
		AA 14d G	5	8: **Reg	0.5 (TR)
	ABC019- ABG716 (1.4 cM)	GP 0d F	7	10: *SIM	3.6 (TR)
2HC	MWG865	SSc 0d G	6	9: *SIM	0.4 (TR)
		AA 0d G	4	5: *Reg	0.5 (TR)
3HL	ABG609B- MWG838 (5.8 cM)	SSc 0d G	13	18: **SIM/CIM	0.5 (TR)
		SSc 14d G	3	5: *Reg	0.3 (TR)
		AA 0d G	7	10: *SIM	0.6 (TR)
5HL	MWG602- ABC718 (0.7 cM)	AA 14d G	4	5: *Reg	0.4 (TR)
		SSc 0d G	12	16: **SIM	0.5 (TR)
		SSc 0d F	17	28: **SIM	0.5 (TR)
		SSc14d F	5	7: **Reg	0.3 (TR)
		AA 0d G	7	10: **Reg	0.6 (TR)
		AA 0d F	8	12: *SIM	1 (TR)
		AA 14d F	3	5: *Reg	0.4 (TR)
7HS	dRpg1- ABG077 (0.7 cM)	GP 0d G	40	71: **SIM	12.3 (TR)
		GP 14d G	8	12: *SIM	3 (TR)
		SSc 0d F	4	6: *Reg	0.3 (H)
7HC	MWG003- Ris 15 (18.9 cM)	AA 0d F	6	9: *SIM	0.8 (H)
		SSc 14d G	6	10: *CIM	0.4 (TR)
		AA 14d G	4	6: *Reg	0.5 (TR)
		GP 14d G	7	11: *SIM/**CIM	2.7 (TR)
		GP 14d F	3	5: *Reg	0.3 (TR)

^a Traits: Preharvest sprouting—SSc sprout score, AA α -amylase activity; Dormancy—GP germination percent, G Greenhouse grown, F Field grown

^b Dormant or preharvest sprouting resistant allele additive effects attributed to Harrington (H); TR306 (TR)

*, ** Significance at $P \leq 0.05$, $P \leq 0.01$ respectively; of likelihood ratio statistics (LRS) by simple interval mapping (SIM); composite interval mapping (CIM); regression (Reg) as predicted by MapManager QTX (Manly et al. 2001)

QTL coincides with the major DOR QTL (SD1) identified in the S/M cross (Han et al. 1996, 1999; Ullrich et al. 2008). Both the 3HL and 5HC Dor QTL regions with concomitant statistics identified in T/M in the current study match almost exactly with the results of the T/M DOR QTL analysis previously reported

(Prada et al. 2004). Interestingly, in all studies (Ullrich et al. 1993, 2008; Prada et al. 2004) so far more than one peak has shown in the major 5HC QTL region (Fig. 4c, d), but with the sub-peaks within the area above the threshold statistic. Therefore, only one QTL was counted in these studies. In fine-mapping this

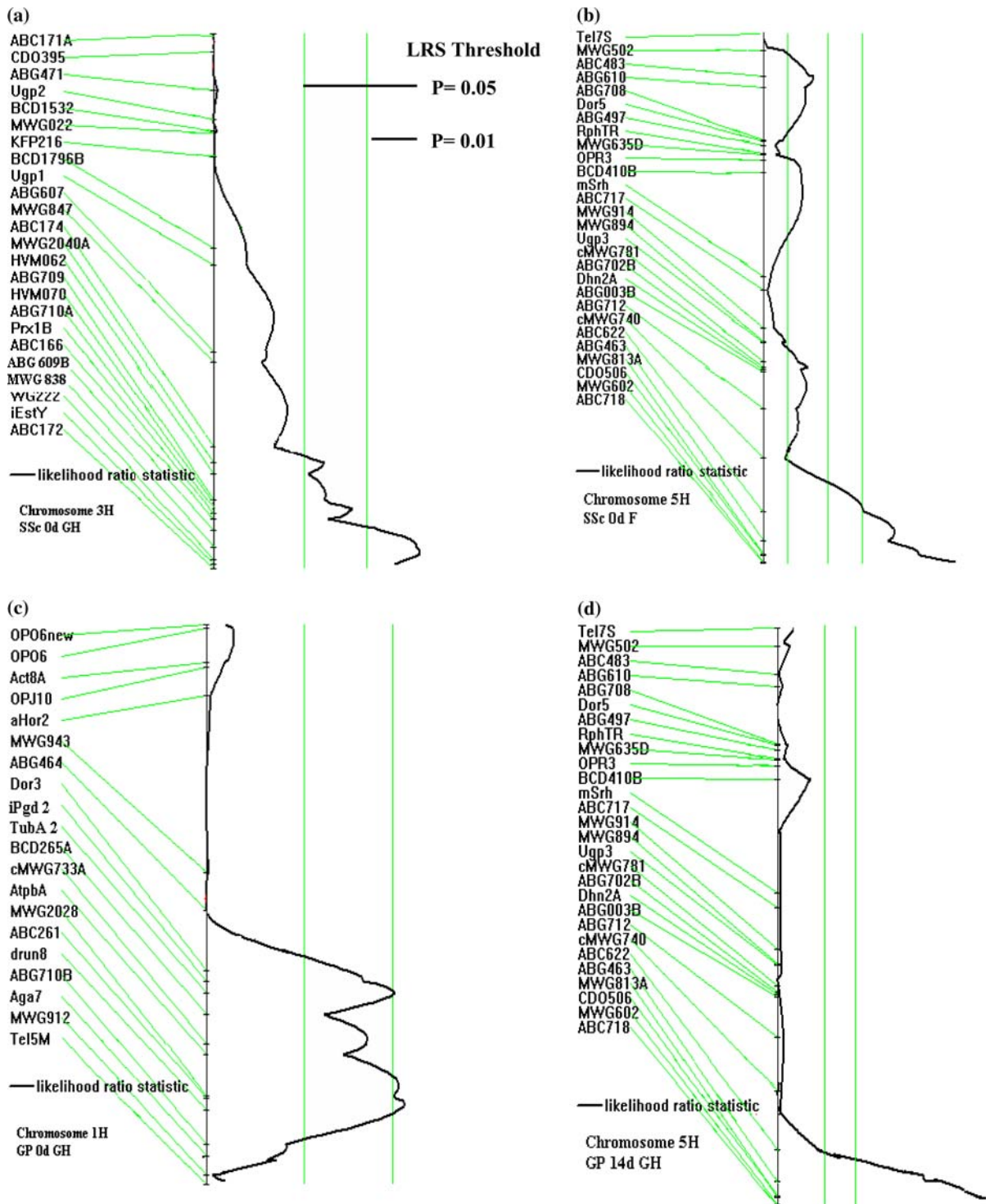


Fig. 3 Likelihood Ratio Statistic (LRS) plots based on simple or composite interval mapping with MapManager QTX of the Harrington/TR306 doubled haploid mapping population for **(a)** sprout score (SSc) at 0-day post-physiological maturity (0d)

from the greenhouse environment (GH) on chromosome 3H; **b** SSc at 0d from field environment (F) on chromosome 5H; **c** germination percentage (GP) at 0d from GH on chromosome 1H; **d** GP at 14d from GH on chromosome 5H

Table 3 QTL regions affecting preharvest sprouting and dormancy in the Triumph/Morex (T/M) cross

Chromosomes	Peak markers/flanking markers	Traits ^a	%Variation explained	LRS significance	Additive effects ^b
1HS	GMS21	SSc 0d	4	4: *Reg	0.2 (M)
		SSc 14d	5	5: *Reg	0.2 (M)
		AA 0d	10	10: **SIM	0.4 (M)
		GP 0d	6	6: *Reg	5.4 (M)
		GP 14d	5	5: *Reg	9.9 (M)
3HL	E39M49_j– E39M48_c (20.6 cM)	SSc 14d	5	5: *Reg	0.2 (M)
		AA 14d	5	4: *Reg	0.4 (M)
		GP 0d	7	6: *Reg	4.6 (M)
5HC	E39M49_f– MWG522 (75 cM)	GP 14d	13	12: **SIM/CIM	13.7 (M)
		SSc 0d	6	6: *Reg	0.2 (T)
		SSc 14d	33	42: **SIM	0.4 (T)
		AA 0d	11	12: *SIM/CIM	0.4 (T)
7HC	E32M48_c– E39M48_p (11.7 cM)	AA 14d	38	50: **SIM	1.1 (T)
		GP 0d	14	15: *SIM	6.8 (T)
		GP14d	54	80: **SIM	28 (T)
7HL	E37M60_g	SSc 0d	4	4: *Reg	0.2 (M)
		GP14d	7	17: **CIM	8.8(M)
7HL	E37M60_g	SSc 0d	7	8: **Reg	0.2 (M)
		GP14d	6	13: **CIM	8.6 (M)

^a Traits: Preharvest sprouting—SSc sprout score, AA α -amylase activity; Dormancy—GP germination percent

^b Dormant or preharvest sprouting resistant allele additive effects attributed to Triumph (T); Morex (M)

*,** Significance at $P \leq 0.05$, $P \leq 0.01$ respectively; of likelihood ratio statistics (LRS) by simple interval mapping (SIM); composite interval mapping (CIM); regression (Reg) as predicted by MapManager QTX (Manly et al. 2001)

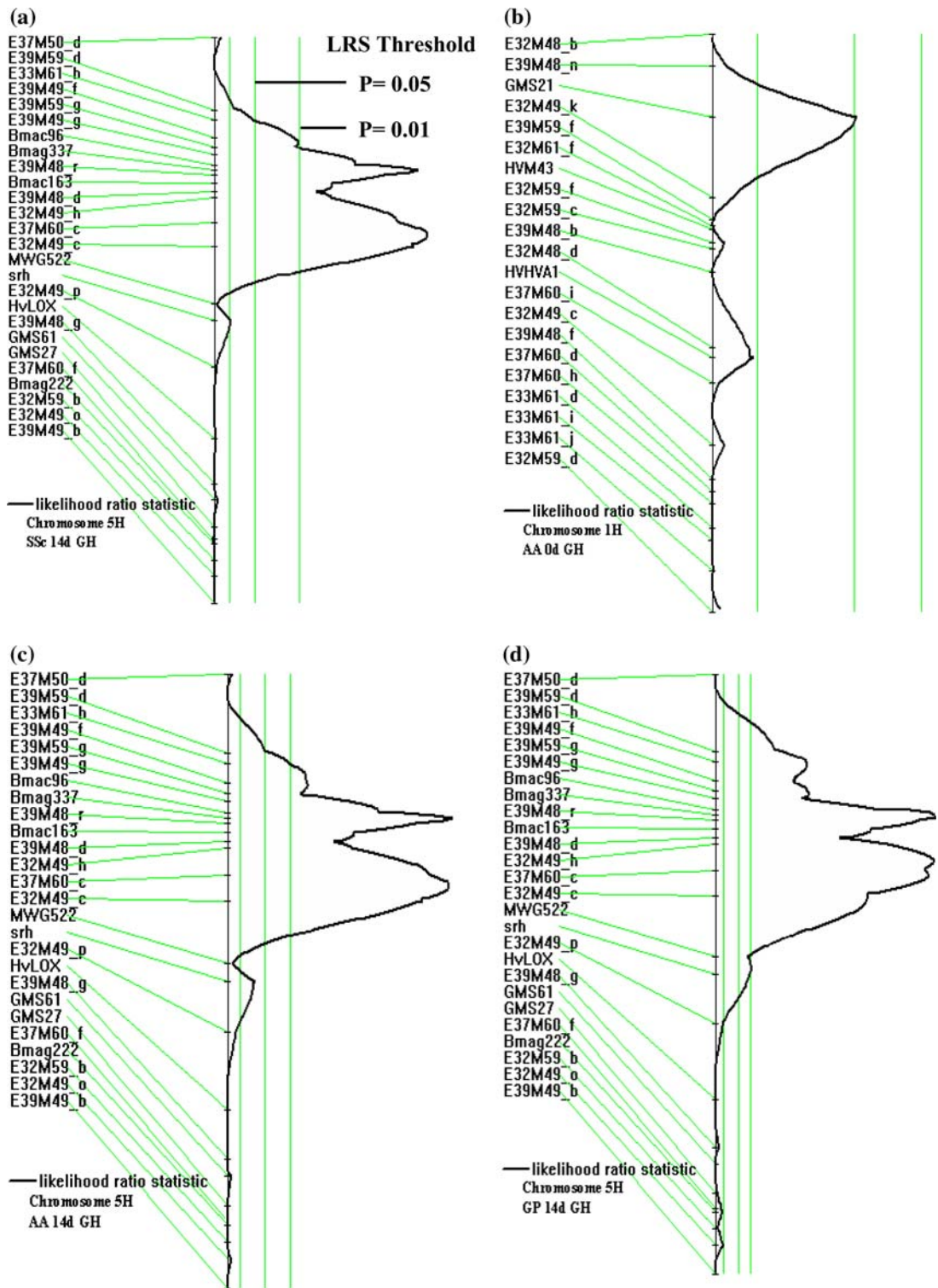
QTL region with S/M isolines, a cluster of at least three genes was indicated (Han et al. 1999), which may explain the occurrence of multiple peaks in the QTL plots.

Allelic effects of selected QTLs

In order to test the accuracy and validity of QTL analyses in this study, trait means of map population DHLs with alternate alleles (H verses TR; T verses M) at the QTL peak marker (highest LRS) of a sample of SSc and GP QTLs displaying a range of effects (R^2 6–54%) were calculated. The differences in means for the various allele comparisons were very clear cut and matched the expected high verses low values based on the designations of the contributing parents as dormant/non-dormant or preharvest sprouting resistant/susceptible (Tables 4 and 5). For example in the H/TR population, for marker MWG838 on chromosome 3HL, DHL SSc 0d GH means for the

Harrington and TR306 alleles were 1.9 ± 0.04 and 0.9 ± 0.04 , respectively (Table 4). This QTL affects 13% of the variation and TR306 contributed the PHS resistant allele. Therefore, it is expected that the DHLs with the TR306 allele would have a lower SSc than those with the Harrington allele. In the case of marker MWG602 on chromosome 5HL, there was a large difference in DHL GP 0d GH means between the two alleles: DHL GP mean with the Harrington allele was 91.2 ± 3.3 and with TR306, 66.5 ± 3.9

Fig. 4 Likelihood Ratio Statistic (LRS) plots based on simple or composite interval mapping with MapManager QTX of the Triumph/Morex doubled haploid mapping population for (a) sprout score (SSc) at 14-day post-physiological maturity (14d) from the greenhouse environment (GH) on chromosome 5H; (b) alpha-amylase activity (AA) at 0-day post-physiological maturity (0d) from GH on chromosome 1H; (c) AA at 14d from GH on chromosome 5H; (d) germination percentage (GP) at 14d from GH on chromosome 5H



(Table 4). This major QTL affects 40% of the variation and TR306 contributed the dormant allele. In the T/M population and the case of the non-dormant

parent, Morex, contributing a dormant allele, for marker E39M49_j on chromosome 3HL, DHL GP 14d means for the Triumph and Morex alleles were

Table 4 Double haploid line means \pm standard errors of sprout score (SSc) and germination percentage (GP) of Harrington (H) or TR306 (TR) allele at selected QTL markers from 0d and 14d post-physiological maturity in greenhouse (G) and field (F)

Chromosome	QTL peak marker	Trait	%Variation explained	Means of each allele type	Additive effect ^a
1HL	MWG2028	GP 0d G	10	H: 74.1 \pm 3.4	6.3 (H)
				TR: 85.5 \pm 3.8	
	ABC261	GP 0d G	10	H: 72.5 \pm 3.6	6.1 (H)
				TR: 84.0 \pm 3.7	
2HS	ABG058	SSc 14d G	7	H: 2.1 \pm 0.4	0.4 (TR)
				TR: 1.5 \pm 0.4	
	ABG019	GP 0d F	7	H: 86.6 \pm 3.4	3.6 (TR)
				TR: 79.7 \pm 3.0	
3HL	MWG838	SSc 0d G	13	H: 1.9 \pm 0.4	0.5 (TR)
5HL	MWG602	SSc 0d G	12	H: 2.0 \pm 0.4	0.5 (TR)
				TR: 0.9 \pm 0.3	
		SSc 0d F	17	H: 2.5 \pm 0.4	0.5 (TR)
				TR: 1.4 \pm 0.4	
7HC	MWG626	GP 0d G	40	H: 91.2 \pm 3.3	12.3 (TR)
				TR: 66.5 \pm 3.9	
		GP 14d G	8	H: 96.7 \pm 1.1	2.9 (TR)
				TR: 90.5 \pm 2.1	
		GP 14d G	7	H: 97.9 \pm 1.4	2.7 (TR)
				TR: 92.4 \pm 1.8	

^a Dormant or preharvest sprouting resistant allele additive effects attributed to H; TR as predicted by MapManager QTX (Manly et al. 2001)

34.0 \pm 5.1 and 14.8 \pm 4.3, respectively (Table 5). These results confirm the QTL determinations and their effects based on SIM and/or CIM analyses. With one exception (2HS SSc 14d GH QTL marked by ABG058 in H/TR, Table 4), all allele mean differences were clearly outside the SEs. The data presented in Tables 4 and 5 also verify validity of the use of these markers in marker assisted selection in breeding for a balance between dormancy and preharvest sprouting resistance.

Summary and conclusions

Interval (SIM/CIM) mapping has revealed nine QTL regions on five chromosomes that affect PHS and/or DOR traits from the H/TR cross (Table 2) and five regions on four chromosomes from the T/M cross (Table 3). Previously from the S/M cross, eight PHS

and/or DOR QTL regions on six chromosomes were identified (Ullrich et al. 2008), from the Stirling/Harrington cross, seven DOR QTL regions on five chromosomes were identified (Bonnardeaux et al. 2008), from the T/M cross, four DOR QTL regions on three chromosomes were detected (Prada et al. 2004), and from the Chebec/Harrington cross, four PHS and/or Dor QTL regions on three chromosomes were detected (Li et al. 2003). Many of the above cited chromosome regions had PHS and DOR QTLs coincident. In the present study from the H/TR cross, six QTL regions affected PHS and five regions affected DOR, with two of the regions affecting both PHS and DOR (on chromosomes 5HL and 7HC). Four chromosome regions affected only PHS and three affected only DOR. From the T/M cross, two QTL regions affected PHS, four affected DOR with one of the regions affecting both traits (on chromosome 5HC). From the S/M cross, seven of eight QTL regions

Table 5 Double haploid line mean \pm standard error of sprout score (SSc) and germination percentage (GP) of Triumph (T) or Morex (M) allele at selected QTL markers from 0d and 14d post-physiological maturity in greenhouse

Chromosome	QTL peak marker	Trait	%Variation explained	Means of each allele type	Additive effect ^a
3HL	E39M49_j	GP 14d	13	T: 34 \pm 5.1 M: 14.8 \pm 4.3	13.7 (M)
5HC	E37M60_c	SSc 14d	33	T: 0.1 \pm 0.02 M: 0.8 \pm 0.19	0.4 (T)
	Bmag337	GP 14d	54	T: 8.2 \pm 2.1 M: 60.8 \pm 5.6	28 (T)
7HC	E32M49i	GP 14d	7	T: 32.0 \pm 5.2 M: 17.7 \pm 4.8	8.8 (M)
7HL	E37M60_g	GP 14d	6	T: 33.2 \pm 5.1 M: 13.4 \pm 4.4	8.6 (M)

^a Dormant or preharvest sprouting resistant allele additive effects attributed to H; TR as predicted by MapManager QTX (Manly et al. 2001)

affected both PHS and DOR (Ullrich et al. 2008). The above summaries were based strictly on interval mapping results. If regression analysis results are taken into account, the picture is somewhat clouded in some instances. From the T/M cross, the PHS (AA) QTL region on chromosome 1HS has REG significant SSc and DOR (GP) QTLs coincident, and three DOR (GP) QTL regions (on chromosomes 3HL, 7HC, 7HL) have significant REG AA and/or SSc QTLs coincident (Table 3). Therefore, these seemingly unique QTLs for PHS or DOR may not be so unique.

The inclusion of REG-identified QTLs with interval mapped QTL regions helps clarify, as well as, cloud the genetic picture of these related quantitatively inherited traits. Given that the phenotype data include two sub-traits (SSc, AA) for measuring PHS, two maturity/after-ripening periods, and two growing environments, the REG results may fill in gaps. In a perfect world, all SSc QTLs might be coincident with AA QTLs, and 0d and 14d measurements and GH and F environments might give the same results. Of course, these kinds of consistency or agreements don't always occur, and they might not occur anyway, because of the influence of the different "treatments" (maturity, environment, etc.) on a variable number of genes in a given chromosome interval. However, considering the chromosome 1HL MWG2028—Aga7 interval in H/TR, a clearer reality might be realized by the REG SSc QTL showing up with SIM/CIM GP QTL (Table 2). Is there strictly a gene affecting DOR only or does this chromosome 1HL gene or genes

potentially affect PHS directly, as well? In the T/M cross, the chromosome 5HC region includes QTLs detected from all six measurements (0 and 14d—SSc, AA, GP) if the SSc 0d REG result is included (Table 3). This completes a perfectly consistent PHS/DOR QTL picture.

Overall, from the various studies on PHS and DOR in barley and wheat, a relatively large number of QTLs, and therefore genes, spread over most of the chromosomes in the genomes affect these two traits. There are a few QTLs or genes with major impacts, but the traits are influenced by potentially many genes, as there is a wide range of effects among the QTLs identified to date. The complexity of inheritance is supported further by continuous frequency distributions of the traits in this (Figs. 1 and 2.) and other barley studies as cited above and other studies in wheat (Paterson and Sorrells 1990; Anderson et al. 1993). Evidence for additivity and epistasis among large and small effect QTLs for DOR has been cited in previous studies in barley and wheat (Anderson et al. 1993, Romagosa et al. 1999; Kulwal et al. 2005; Mares et al. 2005).

There is considerable overlap of QTLs for PHS and DOR, which may indicate that these QTLs control both traits represented by alternative alleles at the same locus explaining the reason dormancy has been viewed opposite to PHS. But perhaps more importantly, there seem to be unique QTLs (genes) for one or the other trait. Conservatively including REG QTL results, from the H/TR cross on chromosomes 2HS,

2HC, 3HL and 7HS, unique PHS QTLs were revealed, as well as, unique DOR QTLs on chromosomes 1HL and 2HS (Table 3). A unique DOR QTL was identified on chromosome 1HL from S/M as well (Ullrich et al. 2008). QTL regions that seem to affect just one or the other trait may suggest that the two traits are not completely opposite and not always represented by alternate alleles at a given locus. The evidence does point to partial independent genetic control. Still these traits are physiologically and genetically associated, but seemingly not completely.

Among the various studies of DOR and PHS in barley, the 5HC and 5HL telomere QTLs identified in this study (Tables 3 and 4) are the most conserved and exert the greatest effects. However, there are other more minor QTLs that are conserved across genotypes, as mentioned above on chromosomes 2HS and 7HC. The 5HC QTL region (=SD1 in S/M) contains both DOR and PHS genes in the T/M and S/M crosses (Ullrich et al. 1993, 2008; Han et al. 1996, 1999; Prada et al. 2004). The 5HL telomere QTL region (=SD2 in S/M) also contains DOR and PHS genes detected in H/TR (Takeda 1996; Ullrich et al. 2002), S/M, (Ullrich et al. 1993, 2008; Han et al. 1996; Gao et al. 2003), Chebec/Harrington (Li et al. 2003), and Stirling/Harrington (Bonnardeaux et al. 2008). Furthermore, these two chromosome 5H QTL regions acted additively in the S/M population in determining the time of dormancy release (Romagosa et al. 1999). Also it has been proposed (Li et al. 2004) that the chromosome 5HL telomere region may involve a gene encoding gibberellin (GA) 20-oxidase which is known to play a role during dormancy release (Adkins et al. 2002). The telomere region of chromosome 5HL also controls malting quality traits, such as malt extract, diastatic power, malt beta-glucan content, and soluble protein with PHS susceptible Harrington contributing the high quality alleles (Mather et al. 1997). Thus it may be difficult to control PHS through chromosome 5HL manipulation in at least two-row malting barley without compromising malting quality. On the other hand, given the number and variable effects of QTLs for malting quality, PHS, and DOR and potential independence of some PHS and DOR QTLs, it may be possible to successfully manage PHS in malting barley.

This study and others continue to add to the body of knowledge about the inheritance of PHS and DOR and their relationships. The identification of molecular

markers linked to chromosome regions that affect these traits present opportunities for marker assisted selection for further genetic study and breeding improved cultivars. A moderate level of dormancy or preharvest sprouting resistance is desirable for commercial production and end use of barley.

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References

- Adkins SW, Bellairs SM, Loch DS (2002) Seed dormancy mechanisms in warm season grass species. *Euphytica* 126:13–20
- Anderson JA, Sorrells ME, Tanksley SD (1993) RFLP analysis of genomic regions associated with resistance to preharvest sprouting in wheat. *Crop Sci* 33:453–459
- Barr AR, Karakousis A, Lance RCM, Logue SJ, Manning S, Chalmers KJ, Kretschmer JM, Boyd WJR, Collins HM, Roumeliotis S, Coventry SJ, Moody DB, Read BJ, Paulsen D, Li CD, Platz GJ, Inkerman PA, Panozzo JF, Cullis BR, Smith AB, Lim P, Langridge P (2003) Mapping and QTL analysis of the barley population Chebec x Harrington. *Aust J Agric Res* 54:1125–1130
- Bewley JD, Black M (1994) *Seeds: Physiology of development and germination*, vol 2. Plenum Press, New York
- Bonnardeaux Y, Li C, Lance R, Zhang X, Sivasithamparam K, Appels R (2008) Seed dormancy in barley: identifying superior genotypes through incorporating epistatic interactions. *Australian J Agric. Res* 59:517–526
- Carn JD (1980) Detection of rain damaged barley at harvest (pre-harvest sprouting). In *Proceedings of 30th Annual Australian cereal Chemistry Conference RACI, Melbourne*
- DePauw RM, McCaig TN (1991) Components of variation, heritabilities and correlations for indices of sprouting tolerance and seed dormancy in *Triticum* spp. *Euphytica* 52:221–229
- Gao W, Clancy JA, Han F, Prada D, Kleinhofs A, Ullrich SE (2003) Molecular dissection of a dormancy QTL region near the chromosome 7 (5H) L telomere in barley. *Theor Appl Genet* 107:552–559
- Han F, Ullrich SE, Clancy JA, Jitkov VA (1996) Verification of barley seed dormancy loci via linked molecular markers. *Theor Appl Genet* 92:87–91
- Han F, Ullrich SE, Clancy JA, Romagosa I (1999) Inheritance and fine mapping of a major barley seed dormancy QTL. *Plant Sci* 143:113–118
- Hori K, Sato K, Takeda K (2007) Detection of seed dormancy QTL in multiple mapping populations derived from crosses involving novel barley germplasm *Theor Appl Genet* 115:869–876
- Kasha KJ, Kleinhofs A, Kilian A, Saghai-Marooof M, Scoles GJ, Hayes PM, Chen FQ, Xia X, Li X-Z, Biyasev RM, Hoffman D, Dahleen L, Blake TK, Rossnagel BG, Steffenson

- BJ, Thomas PL, Falk DE, Laroche A, Kim W, Molnar SJ, Sorrells ME (1995) The North American barley map on the HT cross and its comparison on the cross SM. In: Tsunewaki K (ed) *The plant genome and plastome: their structure and evolution*. Kodansha Scientific Ltd, Tokyo, pp 73–88
- Kato K, Nakamura W, Tabiki T, Miura H, Sawada S (2001) Detection of loci controlling seed dormancy on group 4 chromosomes of wheat and comparative mapping with rice and barley genomes. *Theor Appl Genet* 102:980–985
- King RW, von Wettstein-Knowles P (2000) Epicuticular waxes and regulation of ear wetting and pre-harvest sprouting in barley and wheat. *Euphytica* 112:157–166
- Kulwal PL, Kumar N, Gaur A, Khurana P, Khurana JP, Tyagi AK, Balyan HS, Gupta PK (2005) Mapping a major QTL for pre-harvest sprouting tolerance on chromosome 3A in bread wheat. *Theor Appl Genet* 111:1052–1059
- Li CD, Tarr A, Lance RCM, Harasymow S, Uhlmann J, Westcot S, Young KJ, Grime CR, Cakir M, Broughton S, Apples R (2003) A major QTL controlling seed dormancy and pre-harvest sprouting/grain α -amylase in two-rowed barley (*Hordeum vulgare* L.). *Australian J Agric. Res* 54:1303–1313
- Li CD, Ni P, Francki M, Hunter A, Zhang Y, Schibeci D, Li H, Tarr A, Wang J, Cakir M, Yu J, Bellgard M, Lance R, Appels R (2004) Genes controlling seed dormancy and pre-harvest sprouting in a rice-wheat-barley comparison. *Funct Integr Genomics* 4:84–93
- Manly KF, Cudmore RH Jr, Meer JM (2001) Map Manager QTX, cross platform software for genetic mapping. *Mamm Genome* 12:930–932
- Mares DJ (1989) Pre-harvest sprouting damage and sprouting tolerance: assay methods and instrumentation. In: Derera NF (ed) *Pre-harvest field sprouting in cereals*. CRC Press Inc., Boca Raton, Florida, pp 129–170
- Mares D, Mrva K, Cheong J, Williams K, Watson B, Storlie E, Sutherland M, Zou Y (2005) A QTL located on chromosome 4A associated with dormancy in white- and red grained wheats of diverse origin. *Theor Appl Genet* 111:1357–1364
- Mather DE, Tinker NA, Laberge DE, Edney M, Jones BL, Rosnagel BG, Legge WG, Briggs KG, Irvine RB, Falk DE, Kasha KJ (1997) Regions of genome that affect grain and malt quality in a North American two-row barley cross. *Crop Sci* 37:544–554
- Paterson AH, Sorrells ME (1990) Inheritance of grain dormancy in white kernelled wheat. *Crop Sci* 30:25–30
- Prada D, Molina-Cano JL, Ullrich SE, Clancy JA, Cistue L, Romagosa I (2004) Genetic control of dormancy in a Triumph/Morex cross in barley. *Theor Appl Genet* 109:62–70
- Romagosa I, Han F, Clancy JA, Ullrich SE (1999) Individual locus effects on dormancy during seed development and after ripening in barley. *Crop Sci* 39:74–79
- Takeda K (1996) Varietal variation and inheritance of seed dormancy in barley. In: Noda K, Mares D (eds) *Preharvest sprouting in cereals 1995*. Cen. Acad. Soc, Osaka, pp 205–212
- Ullrich SE, Hays PM, Dyer WE, Black TK, Clancy JA (1993) Quantitative trait locus analysis of seed dormancy in Steptoe barley. In: Walker-Simmons MK, Ried JL (eds) *Preharvest sprouting in cereals 1992*. Am Assoc Cereal Chem, St Paul, pp 136–145
- Ullrich SE, Han F, Gao W, Prada D, Clancy JA, Kleinhofs A, Romagosa I, Molina-Cano JL (2002) Summary of QTL analyses of the seed dormancy trait in barley. *Barley Newsletter*. 45: 39–41. Available at: <http://wheat.pw.usda.gov/ggpages/BarleyNewsletter/45/Proceedings1.html>
- Ullrich SE, Clancy JA, del BIA, Lee H, Jitkov A, Han F, Kleinhofs A, Matsui K (2008) Genetic analysis of pre-harvest sprouting in barley. *Mol Breeding* 21:249–259
- Zhang F, Chen G, Huang Q, Orion O, Krugman T, Fahima T, Korol AB, Nevo E, Gutterman Y (2005) Genetic basis of barley caryopsis dormancy and seedling desiccation tolerance at the germination stage. *Theor. Appl. Genet.* 110:445–453