

Genetic analysis of preharvest sprouting in a six-row barley cross

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Abstract Preharvest sprouting (PHS) can be a problem in barley (*Hordeum vulgare* L.) especially malting barley, since rapid, uniform, and complete germination are critical. Information has been gained by studying the genetics of dormancy (measured as germination percentage, GP). The objective of this study was to determine if the quantitative trait loci (QTLs) discovered in previous research on dormancy are related to PHS. PHS was measured as sprout score (SSc) based on visual sprouting in mist chamber-treated spikes and as alpha-amylase activity (AA) in kernels taken from mist chamber-treated spikes that showed little or no visible sprouting. GP was also measured. All traits were measured at 0 and 14 days after physiological maturity. Evaluation of the spring six-row cross, Steptoe (dormant)/Morex (non-dormant) doubled haploid mapping population grown in greenhouse and field environments revealed QTL regions for SSc, AA, and GP on five, four, and six of the seven barley chromosomes, respectively. In total, seven and eight regions on five and six chromosomes had effects ranging from 4 to 31% and 3 to 39% on PHS and dormancy, respectively. One chromosome 3H and three

chromosome 5H QTLs had the greatest effects. All PHS QTLs coincide with known dormancy QTLs, but some QTLs appear to be more important for PHS than for dormancy. Key QTLs identified should benefit breeding of barley for a suitable balance between PHS and dormancy.

Keywords Barley · Dormancy · Germination · *Hordeum vulgare* · Preharvest sprouting · QTL analysis

Abbreviations

AA	Alpha-amylase activity
DH	Doubled haploid
GP	Germination percentage
LRS	Likelihood ratio statistic
PHS	Preharvest sprouting
PM	Physiological maturity
QTL	Quantitative trait locus
S/M	Steptoe/Morex
SSc	Sprout score

Introduction

Preharvest sprouting (PHS) can be a problem in barley (*Hordeum vulgare* L.) production, especially in malting barley (Schwarz et al. 2004). Rain or even very high humidity from near physiological maturity (PM) onward can cause sprouting in the head of standing plants in the field. This has very

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serious consequences for malting grain, since rapid, uniform, and complete germination is critical in the malthouse. Seed dormancy defined as the failure of viable mature seed to germinate under favorable conditions can also be a problem. Dormancy is sometimes viewed as the opposite trait to PHS. In fact, dormancy has been defined as resistance to PHS (Barr et al. 2003). However, it is not clear whether dormancy and PHS are represented by opposite alleles for a given gene.

Dormancy/non-dormancy is generally inferred by measuring germination percentage. PHS, on the other hand, is generally measured more directly by visual sprout score (SSc) and/or alpha-amylase activity (AA) directly or indirectly (e.g., falling number score). Both dormancy and PHS may be measured at some time or times from PM for up to several weeks of after-ripening.

Dormancy quantitative trait loci (QTLs) were previously mapped in several barley crosses including the US Barley Genome Project crosses of ‘Steptoe’/‘Morex’(S/M) (Ullrich et al. 1993; Han et al. 1996, 1999; Gao et al. 2003), and ‘Harrington’/TR306 (Takeda 1996; Ullrich et al. 2002), as well as, ‘Chebec’/Harrington (Li et al. 2003), ‘Triumph’/Morex (Prada et al. 2004), and ‘Mona’/ Wadi Qilt 23–39 (*Hordeum vulgare spontaneum*) (Zhang et al. 2005).

Dormancy QTLs have been mapped to all seven barley chromosomes. Relatively, major effect (>10% variation explained) and/or consistent QTL regions have been identified across environments and genotypes/crosses on chromosomes 1H, 4H, 5H, and 7H (Ullrich et al. 1993; Han et al. 1996; Takeda 1996; Li et al. 2003; Prada et al. 2004). Specifically in the S/M cross, one relatively consistent QTL region each was identified on chromosomes 4H, 7H, and two on 5H (Ullrich et al. 1993; Han et al. 1996).

Relatively little research has been done to characterize the genetics of PHS potential per se in barley. QTL analysis of PHS as measured by AA after significant rainfall near harvest of a field grown Chebec/Harrington mapping population revealed a major effect QTL near the telomere of chromosome 5HL and a minor effect one on chromosome 2H (Li et al. 2003, 2004). King and Wettstein-Knowles (2000) showed that morphological features of epicuticular waxes and spike angle at maturity can affect ear wetting and PHS in barley and wheat (*Triticum aestivum* L. and *T. durum* L.).

An objective of a major ongoing study in our program is to determine if the barley germplasm developed and QTLs discovered in previous research on dormancy can be applied or related to the genetic control of PHS. This paper reports results from the S/M cross, which represents dormant (Steptoe) and non-dormant (Morex) six-row spring types. Our hypothesis was that some PHS QTLs will be the same as previously determined dormancy QTLs and some PHS QTLs will be different from or more important than dormancy QTLs.

Materials and methods

Grain production

The Steptoe (dormant type)/Morex (non-dormant type) mapping population consisting of 150 F₁-derived doubled haploid (DH) lines, the parents, and molecular marker map developed from this population were utilized in this study (original: Kleinhofs et al. 1993; current: <http://barleygenomics.wsu.edu/>). The S/M DHs and parents were grown in one greenhouse and one field environment 2003–2005. Standard barley growing conditions of 20/15°C, day/night temperatures and 16 h photoperiod were used in the greenhouse. Standard barley growing conditions and two 3 m long row plots were used in the field at Spillman Agronomy Farm, Pullman, WA. Fifteen heads were harvested of each genotype in both growing environments at PM (when all green color was lost from the head) and 14 days later. All heads were frozen at –20°C immediately after harvest until analyses were performed.

Preharvest sprouting (PHS) simulation and measurement

To determine susceptibility to PHS, a modification of SSc procedures of DePauw and McCaig (1991) were used. Replication of five and ten heads of each line and parent from greenhouse and field, respectively, harvested at 0 and 14-day post-PM were treated in a mist chamber for 5 days or until Morex, the sprout susceptible parent, showed 80% sprouted heads.

The degree of PHS was determined visually and rated on a 0–5 scale; 0 = no sprouting; 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.

Roots and shoots were counted if they were ≥ 2 mm long. Mean SScs of the five or ten heads rated were used for genetic analysis.

Alpha-amylase activity analysis

Alpha-amylase is synthesized de novo at the onset of germination. Therefore, a measure of alpha-amylase enzyme activity gives a measure of germination in seeds even with no visible sprouting. After PHS simulation, all heads were dried at 70°C for 48 h and threshed by hand. Grain of genotypes with mean SScs of ≤ 1 was milled and enzyme activity determined in duplicate to achieve 10% agreement using the Megazyme Alpha-Amylase kit (<http://www.megzyme.com/booklets/KCERA.pdf>). Analyses were performed on greenhouse and field grown material at 0 and 14-day post-PM, respectively.

Germination test

To determine degree of dormancy, two replications of 50 seeds from 0 day and 14 days harvests from each environment were used for germination using standard procedures in Petri dishes with moist filter paper in a germinator set at 20°C for 7 days. Means of the two replications were used for genetic analysis.

Genetic analysis

The mean phenotypic data [SSc, AA, and germination percentage (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). Five hundred permutation tests were performed on the data to establish Likelihood Ratio Statistic (LRS) thresholds at 1 and 5% probability levels. Regression, simple interval mapping (SIM, no background control for other QTLs) and composite interval mapping (CIM, background control for other QTLs) functions were utilized. SIM identified the most significant QTL in a given trait analysis and subsequent QTLs were

usually identified by CIM. SIM and CIM QTL results significant at $P \leq 0.05$ are emphasized. Significant ($P \leq 0.05$) QTLs indicated by regression analysis not confirmed with a significant LRS from interval mapping are treated as indicators of the potential for the existence of minor QTLs only.

Results and discussion

Phenotypic data

Step toe, the dormant and presumed sprout resistant parent and Morex, the non-dormant and presumed sprout susceptible parent behaved as expected for each trait measured. Step toe had zero or low SScs (0–0.2), AA (0.012–0.034 Ug^{-1} flour), and germination percentages (2–21%), while Morex had relatively high levels of these traits (1.1–5 SSc and 58–100% GP), Tables 1 and 2; Fig. 1. AA was not measured for Morex samples, because SScs were above the threshold for AA measurement (≤ 1), and they would have been extremely high. The distribution patterns for the mapping population were continuous with population means between the two parents and ranges extending to or beyond the parents (Tables 1, 2). The frequency distributions for each trait from the field environment display and characterize the continuous distribution of the mapping population (Fig. 1). The distribution patterns from the greenhouse environment (data not shown) are nearly identical to those from the field. The parents and mapping population in this study (Fig. 1c, f) behaved similarly to that in the initial dormancy study in terms of germination percentage distributions (Ullrich et al. 1993). The phenotypes of the parents and the DHLs of the mapping population confirm extensive polymorphism for each trait studied providing excellent potential for mapping relevant QTLs for each of the traits.

QTL analysis

A relatively high number of QTLs were indicated for the various traits spread across all seven barley chromosomes measured by regression analysis (data not shown), which is similar to the results of the initial dormancy study with the S/M population (Ullrich et al. 1993). These results are also similar

Table 1 Preharvest sprouting and dormancy mean \pm SE phenotypic data for parents and doubled haploid (DH) line mapping population, greenhouse environment

Genotype	Physiological maturity (0 day)			14 days after-ripening	
	Sprout score	α -amylase U g^{-1} flour	Germination %	Sprout score	Germination %
Steptoe	0 \pm 0	0.034	8 \pm 4	0.2 \pm 0.2	2 \pm 0
Morex	5 \pm 0	ND ^a	100 \pm 0	4.4 \pm 0.6	100 \pm 0
DH mean	1.7 \pm 0.3	0.494	42.8 \pm 3.4	1.8 \pm 0.4	72 \pm 2.6
DH minimum	0	0.000	0	0	0
DH maximum	5	4.089	100	5	100
DH <i>n</i>	148	53	142	66	145

SE Standard error of the mean

^a Not determined due to sprout score above the threshold for measuring α -amylase activity

Table 2 Preharvest sprouting and dormancy mean \pm SE phenotypic data for parents and doubled haploid (DH) line mapping population, field environment

Genotype	Physiological maturity (0 day)			14 days after-ripening		
	Sprout score	α -amylase U g^{-1} flour	Germination %	Sprout score	α -amylase U g^{-1} flour	Germination %
Steptoe	0 \pm 0	0.012	2 \pm 0	0 \pm 0	0.025	21 \pm 1.0
Morex	1.1 \pm 0.4	ND ^a	58 \pm 2.0	4.2 \pm 0.1	ND ^a	100 \pm 0
DH mean	0.5 \pm 0.1	0.379	17.2 \pm 2.2	2.2 \pm 0.4	0.487	76.4 \pm 2.1
DH minimum	0	0.000	0	0	0.003	2
DH maximum	5	4.039	97	5	3.995	100
DH <i>n</i>	137	112	142	138	39	141

SE Standard error of the mean

^a Not determined due to sprout score above the threshold for measuring α -amylase activity

to the bread wheat situation in which QTLs for PHS/dormancy have been mapped to 20 of the 21 chromosomes (Kulwal et al. 2005). A number of QTL clusters were indicated in the current study, which means that QTLs for different traits overlap one another in the same chromosome region. Within a given cluster, this may indicate that one gene affects more than one trait (pleiotropy) and/or several genes for the given traits are closely linked. Many of the putative QTLs had relatively small effects, but they explained from 3 to 39% of the variation observed for a given trait. This indicates there is considerable complexity in the inheritance of these traits and there are relationships among the traits.

Simple interval mapping and CIM procedures were applied to verify the significance of QTLs indicated by regression analysis. Only those QTL regions that were verified as significant with SIM or CIM were considered as ‘real’ and are listed in

Table 3. This table includes all QTLs significant by regression, as well as, SIM and CIM within each verified chromosome region. However, those QTLs with trait effects significant by regression analysis only are considered as indications of very minor genes. In all cases Morex contributed the alleles for low dormancy (high germination percentage) and high sprouting potential (high SSc), except for the QTL region on chromosome 2H (see below).

Sprout score

Sprout score QTLs verified with SIM or CIM were mapped to chromosomes, 2HS, 3HS, 4HS, 5HC and L and 7HC. The chromosome 2H, 4H, and 7H QTLs had relatively minor effects explaining only 5 or 6% of the variation by SIM/CIM, which translates to 0.4 SSc change (additive value). The chromosome 3H

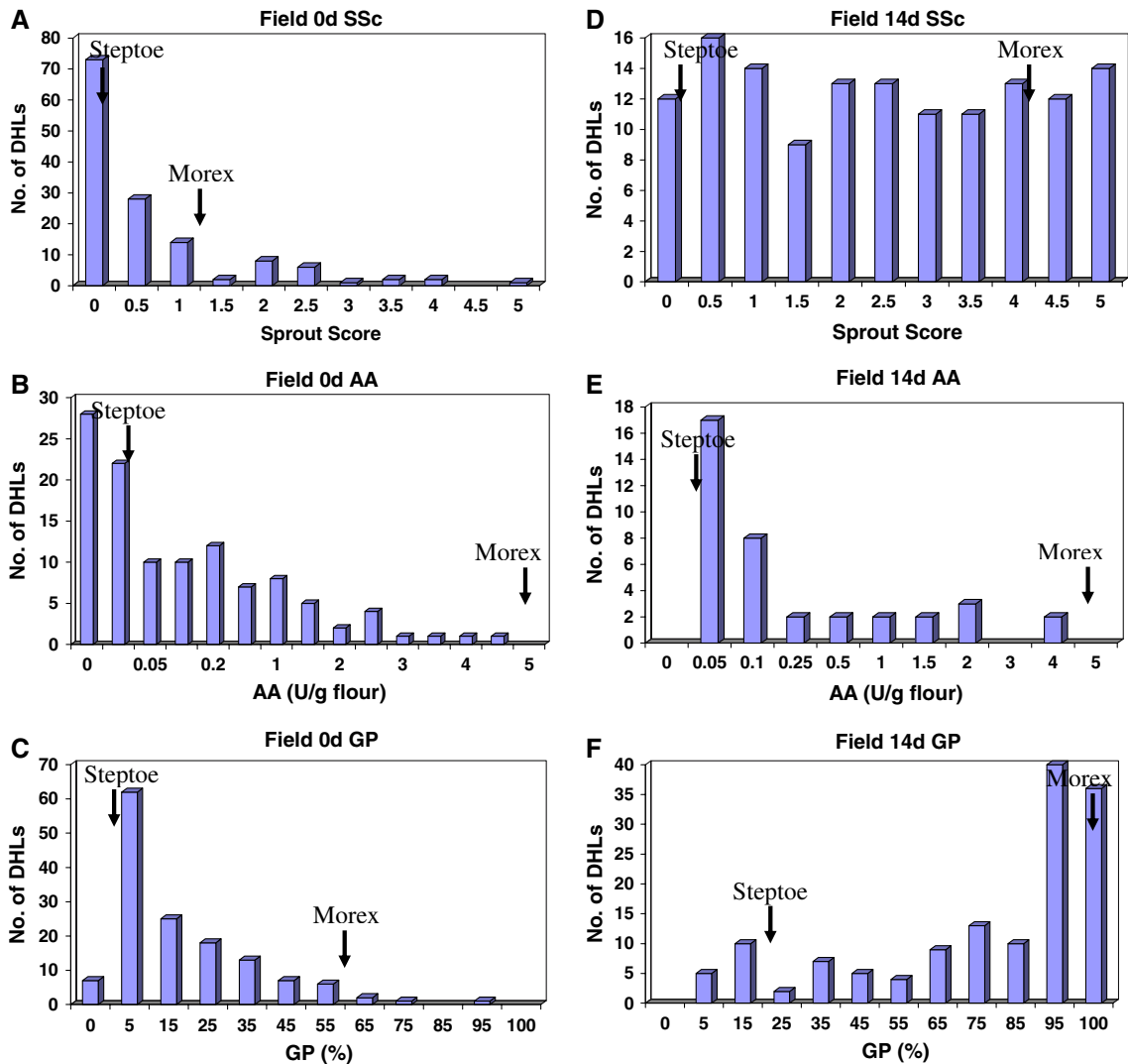


Fig. 1 Frequency distributions for the preharvest sprouting (sprout score, *SSc*; alpha-amylase activity, *AA*) and dormancy (germination percentage, *GP*) traits measured at 0 day (a–c)

and 14 days (d–f) after physiological maturity from the field environment in the Steptoe/Morex doubled haploid mapping population and parents

QTL in the 12.9 cM interval between ABG399A and DD1.1B had SIM/CIM effects ranging from 5 to 15%, which affects up to 0.6 *SSc* (Table 3; Fig. 2a). This corresponds to a very minor dormancy QTL identified from only one of eight environments in the original S/M dormancy study (Ullrich et al. 1993). Whereas, there may be minor dormancy QTL effects in this chromosome 3H region, there seems to be greater effects on PHS. Both PHS and dormancy QTLs have been implicated on the group 3 chromosomes in common wheat, but the long arm seems to

be more important than the short arm in wheat (Miura et al. 2002; Kulwal et al. 2005).

The greatest QTL effects on *SSc* were found on chromosome 5H, specifically in the 9.2, 11.2, and 13.7 cM intervals between CDO348B and ABC302A, WG908 and ABG495A, and ABG463 and Tel7L (note: current map notation = Tel5H), respectively (Table 3; Fig. 2b). The chromosome 5H QTL effects on *SSc* ranged from 6 to 31% determined by SIM/CIM. The QTL in the chromosome 5HC CDO348B–ABC302A interval had the greatest

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

Chromosome	Flanking markers	Trait ^a	% variation explained	Additive value ^b	LRS; significance
1HL	ABC261–AGA 7 (2.7 cM)	GP 14 days F	3	5.3	12; CIM*
2HS ^c	WG516–DAK213A (28.3 cM)	SSc 0 day G	5	−0.4	15; CIM**
		AA 0 day G	7	−0.59	15; CIM*
		AA 14 days F	5	0.49	7; Reg*
		GP 0 day G	7	−9.6	20; CIM**
		GP 14 days G	4	−5.9	10; CIM*
		GP 14 days F	6	7.1	18; CIM**
3HC ^c	ABG399A–DD1.1B (12.9 cM)	SSc 0 day G	15	0.6	37; CIM**
		SSc 0 day F	6	0.3	9; Reg**
		SSc 14 days G	10	0.5	10; CIM*
		SSc 14 days F	5	−0.3	10; CIM*
		AA 0 day G	8	0.69	16; CIM*
		AA 0 day F	3	0.38	4; Reg*
		GP 0 day G	6	8.2	8; Reg**
		GP 0 day F	10	6.0	19; CIM**
4HS ^{c,d}	MWG634–JS 103.3 (11.5 cM)	SSc 0 day G	6	0.4	9; SIM*
		SSc 0 day F	4	0.2	6; Reg*
		SSc 14 days F	5	0.4	7; Reg**
		AA 0 day G	5	0.53	7; Reg**
		AA 0 day F	7	0.54	10; SIM*
		GP 0 day G	6	8.7	9; SIM*
5HC ^c	CDO348B–ABC302A (9.2 cM)	SSc 0 day G	31	0.9	54; SIM**
		SSc 0 day F	11	0.3	16; SIM**
		SSc 14 days G	9	0.6	8; Reg**
		SSc 14 days F	26	0.8	42; SIM**
		AA 0 day G	27	1.19	43; SIM**
		AA 0 day F	7	0.52	9; SIM*
		AA 14 days F	12	0.79	25; CIM**
		GP 0 day G	35	20.9	61; SIM**
		GP 0 day F	16	7.5	24; SIM**
		GP 14 days G	38	19.2	70; SIM**
		GP 14 days F	39	19.5	69; SIM**
5HL ^c	WG908–ABG495A (11.2 cM)	SSc 0 day F	6	0.2	8; SIM*
		SSc 14 days F	10	0.5	21; CIM**
		AA 14 days F	19	0.94	28; SIM**
		GP 0 day F	6	4.7	10; SIM*
5HL ^c	ABG463–Tel7L = 5HL (13.7 cM)	SSc 0 day G	5	0.3	7; Reg*
		SSc 14 days G	16	0.6	12; SIM*
		SSc 14 days F	12	0.6	23; CIM**
		AA 0 day G	5	0.50	11; CIM*
		AA 14 days F	12	0.76	20; CIM**
		GP 0 day G	11	10.9	15; CIM**
		GP 14 days G	13	11.2	33; CIM**
		GP 14 days F	18	12.6	46; CIM**

Table 3 continued

Chromosome	Flanking markers	Trait ^a	% variation explained	Additive value ^b	LRS; significance
7HC ^{c,d}	Brz–Amy 2 (30.7 cM)	SSc 0 day G	6	0.4	18; CIM**
		SSc 14 day G	6	0.4	4; Reg*
		GP 0 day G	4	6.9	5; Reg*
		GP 14 days F	5	5.1	11; CIM**

^a Traits: preharvest sprouting–SSc sprout score, AA α -amylase activity; dormancy—GP germination percent, G greenhouse grown, F field grown

^b Additive value: additive QTL affect in units of measurement; positive and negative values attributed Morex and Steptoe alleles, respectively

^c Coincides with previously mapped dormancy QTL in S/M (Ullrich et al. 1993)

^d Coincides with previously mapped malt AA QTL in S/M (Han and Ullrich 1994)

*, **Significance: $P \geq 0.05$ and 0.01 , respectively; of likelihood ratio statistic (LRS) by simple interval mapping (SIM); composite interval mapping (CIM); *Regression (Reg)*

effect explaining up to 31% of the variation with an additive affect of 0.9 sprout score (SSc 0 day greenhouse). The effects of the WG908–ABG495A interval ranged from 6 to 10%. The effects of the chromosome 5HL telomere region interval ranged from 12 to 16% (Table 3). The chromosome 5H QTL regions also had relatively strong QTLs for AA and germination percentage, as well (see below).

Alpha-amylase activity

Alpha-amylase activity QTLs, verified by SIM or CIM, occur on chromosomes 2HS (WG516–DAK 213A), 3HC (ABG399A–DD1.1B), 4HS (MWG634–JS103.3), 5HC (CDO348B–ABC302A), 5HL (WG908–ABG495A), and 5HL (ABG463–Tel7L) with effects explaining 5–27% of the variation or changing enzyme activity by 0.49–1.19 U/g flour (Table 3). The chromosome 2HS, 3HC, and 4HS QTLs are all relatively minor in effect (7–8%), which was the case for SSc and germination percentage in the chromosome 2HS and 4HS intervals, as well. The chromosome 3HC AA QTL was in the same interval as SSc and germination percentage QTLs, but with smaller effects (Table 3). All three chromosome 5H QTL intervals from the centromere to long arm telomere contain relatively major AA QTLs explaining up to 27, 19, and 12% of the variation, respectively. These QTLs had the greatest affect on AA and they also overlap with SSc and germination percentage QTLs. The chromosome 4HS PHS AA

QTL coincides with a previously mapped malt AA QTL (Hayes et al. 1993; Han and Ullrich 1994). These PHS and malt AA QTLs may also coincide with group 4 chromosome dormancy QTLs in common wheat (Kato et al. 2001). All AA QTLs identified in the present study coincide with previously identified dormancy QTLs in the S/M mapping population, but the chromosome 2H, 3H, and 5HL (WG908–ABG495A) dormancy QTLs were very minor and inconsistently expressing in the original studies (Ullrich et al. 1993; Han et al. 1996). In the two-row/two-row Chebec/Harrington cross, Li et al. (2003) identified a major PHS QTL based on AA in the ABG463–Tel7L interval of chromosome 5H.

Germination percentage

Dormancy QTLs as measured by germination percentage were mapped (SIM and/or CIM) to six of the seven barley chromosomes (Table 3). Relatively minor QTLs were indicated on chromosomes 1HL, 2HS, 4HS, 5HL (the WG908–ABG495A interval), and 7HC. These minor QTLs explained from 3 to 7% variation or affected about 5 to 7% germination. The chromosome 4H and 7H QTLs were previously determined to be minor (5% variation explained), but somewhat consistently expressed across environments (Ullrich et al. 1993; Han et al. 1996). These QTLs were named SD3 and SD4 for the chromosome 7H and 4H intervals, respectively (Han et al. 1996). The chromosome 2H and 5HL

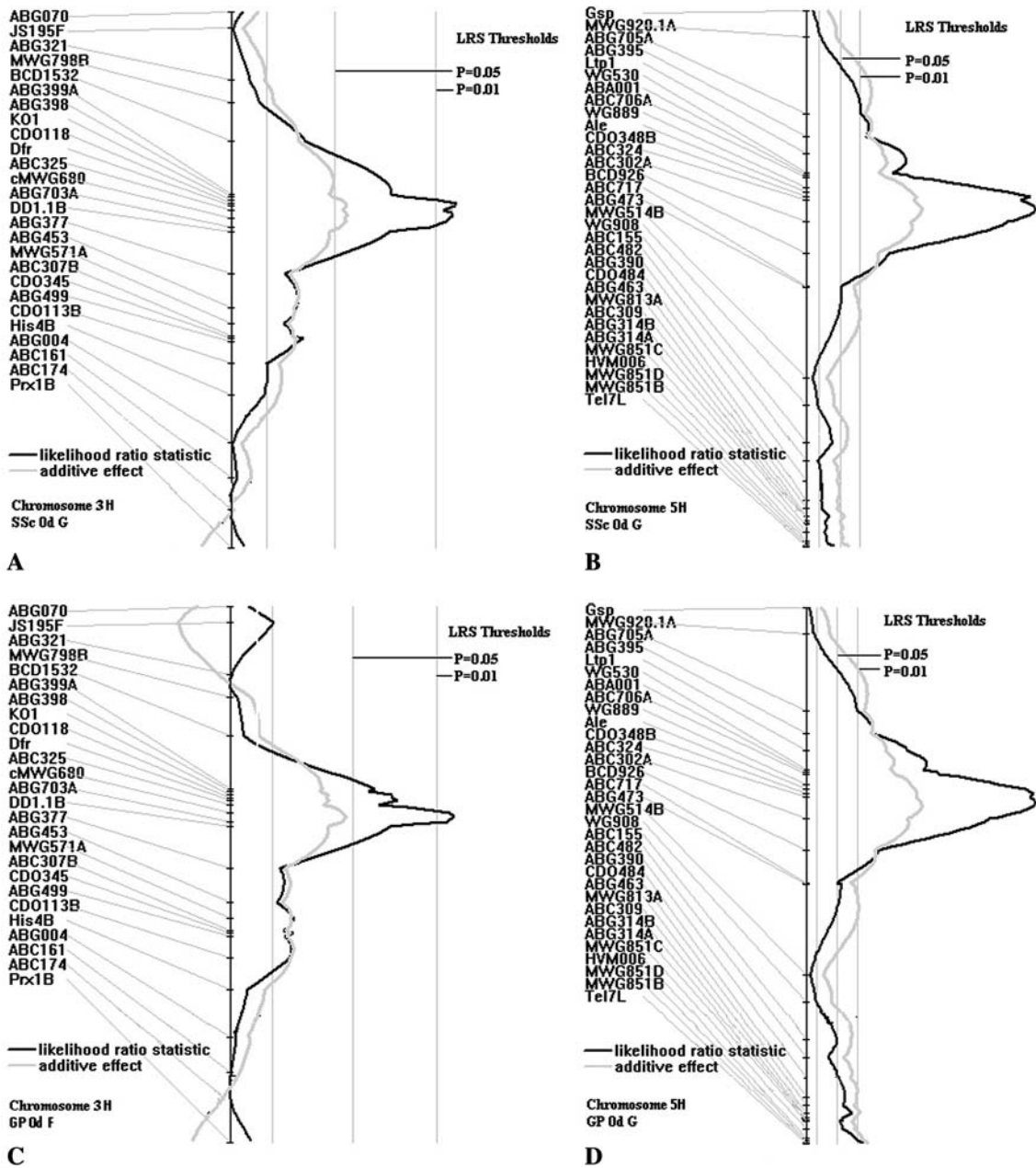


Fig. 2 Likelihood Ratio Statistic (*LRS*) plots based on simple or composite interval mapping with MapManager QTX of the Steptoe/Morex doubled haploid mapping population for (a) sprout score (*SSc*) at 0-day post-physiological maturity (*PPM*) from the greenhouse environment (*G*) on chromosome 3H,

LRS 37 at peak; (b) *SSc* at 0 day *PPM* from *G* on chromosome 5H, *LRS* 54 at peak; (c) germination percentage (*GP*) at 0-day *PPM* from the field environment (*F*) on chromosome 3H, *LRS* 19 at peak; (d) *GP* at 0-day *PPM* from *G* on chromosome 5H, *LRS* 61, and 15 at peaks

(WG908–ABG495A) QTLs were also previously identified as minor, and detected in only one or two of the eight environments evaluated (Ullrich et al. 1993). The very minor QTL on chromosome 1H

was not previously indicated in the S/M cross. The chromosome 3HC QTL region had a 10% effect on dormancy (Table 3; Fig. 2c) and coincided with a PHS QTL as indicated above.

The largest effect QTLs occur on the chromosome 5H intervals, CDO348B–ABC302A with 16–39% effects (which changed germination percentage by about 7–20 points) and ABG463–Tel7L with 11–18% effects (which changed germination percentage by about 11–13 points), Table 3 and Fig. 2d. Again these two chromosome 5H regions coincide with PHS QTLs. These two dormancy QTLs were previously named SD1 (5HL centromere region) and SD2 (5HL telomere region) (Han et al. 1996). These chromosome 5H results are very consistent in terms of location and effect with previous interval mapping (Ullrich et al. 1993; Oberthur et al. 1995), marker assisted selection (Han et al. 1996), and fine mapping (Han et al. 1999; Gao et al. 2003) results in the S/M cross. Furthermore, both chromosome 5H dormancy QTLs were identified in the two-row/six-row Triumph/Morex cross (Prada et al. 2004) and two-row domesticated/two-row wild barley Mona/Wadi Qilt 23–39 (*H. vulgare spontaneum*) cross (Zhang et al. 2005). The ABG463–Tel7L QTL was previously identified in the two-row/two-row crosses of Harrington/TR306 (Takeda 1996; Ullrich et al. 2002) and Chebec/Harrington (Li et al. 2003), as well. Fine mapping of the SD1 QTL region revealed that it potentially is a complex of at least three genes occupying ~4.4 cM between ABC324 and ABC302A (Han et al. 1999). Fine mapping the SD2 QTL revealed its position to be in the 0.7 cM interval between MWG851D and MWG851B very near the long arm telomere (Gao et al. 2003). Romagosa et al. (1999) showed that SD1 was important in determining the time of dormancy release and acts additively with SD2 in the later stages of after-ripening. This information may be relevant in explaining the relationship between dormancy and PHS potential. The SSc QTL in the SD2 QTL region was much more significant at the 14 days after-ripening measurement than the 0 day measurement. Li et al. (2004) found good synteny between the barley chromosome 5HL telomere QTL region with the chromosome 3L telomere region of rice (*Oryza sativa*). This led to the identification of a gene encoding Gibberellic acid-oxidase, which they proposed as a candidate gene for the chromosome 5HL seed dormancy/PHS QTL. Gibberellic acid may be involved in the release of dormancy through promoting the synthesis of hydrolytic and proteolytic enzymes associated with germination (Adkins et al. 2002).

Summary and conclusions

Considering the previously published results and those from this study, some tentative, conclusions may be drawn about the inheritance of PHS susceptibility and resistance and relationship with seed dormancy in barley. Two regions on chromosome 5H near the centromere and long arm telomere play major roles in both PHS potential and dormancy (Table 3; Fig. 2b, d). One or both regions have been consistently implicated in dormancy and/or PHS in all published barley crosses studied (Ullrich et al. 1993, 2002; Takeda 1996; Li et al. 2003; Prada et al. 2004; Zhang et al. 2005).

The chromosome 3H ABG399A–DD1.1B and chromosome 5H WG908–ABG495A QTL regions appear to be more important for control of PHS than for dormancy (Table 3). These regions showed only minor or no dormancy QTL potential in this and previous studies on barley (Ullrich et al. 1993; Han et al. 1996).

In addition to the major QTLs summarized above for PHS and dormancy, a relatively large number of minor QTLs are indicated throughout the genome (Table 3). This appears to be evidence for considerable complexity in the inheritance of these traits. A few major genes may dominate, but are influenced by potentially many other genes. This conclusion is supported by the continuous frequency distributions for the various traits (Fig. 1), as well as similar findings in other studies of these traits in barley, wheat and rice (Paterson and Sorrells 1990; Anderson et al. 1993; Ullrich et al. 1993; Wan et al. 2005). In this study the variation explained by any one QTL was a maximum of 39% and ranged down to 3% (Table 3). Evidence for additivity and epistasis among and between large- and small-effect QTLs for dormancy has been presented in previous studies in barley and wheat (Anderson et al. 1993; Oberthur et al. 1995; Romagosa et al. 1999; Kulwal et al. 2005; Mares et al. 2005).

Preharvest sprouting and dormancy are physiologically related and appear to be genetically related, as well, based on the common map locations in most instances for the two traits (Table 3; Fig. 2). Whereas, linked gene clusters may be an explanation, pleiotropy is just as likely based on the relatedness of traits (Li et al. 2003) and precedence set by fine mapping of related malting quality traits on barley

chromosomes 4H (Gao et al. 2004) and 7H (Han et al. 2004). Actually, these fine mapping studies support both the occurrence of pleiotropy and linked genes in the relationship of related malting quality traits. Some of the overlapping QTLs detected in this study were not always resolved to the exact same peak within the support interval. The effects of a given QTL interval on the various traits were not usually equal either. The flanking marker and support intervals are relatively large for most of the QTLs detected in this study as have been the case in other studies for dormancy and PHS (Ullrich et al. 1993; Li et al. 2003; Prada et al. 2004; Zhang et al. 2005). There is certainly room for multiple genes affecting the same and related traits. Fine mapping of SD1, the largest-effect dormancy QTL in the S/M cross-revealed a cluster of at least three dormancy genes resolved to a 4.4 cM interval within the CDO324–ABC302A interval on chromosome 5H (Han et al. 1999). The fine mapping of SD2, the second largest-effect dormancy QTL in the S/M cross-near the chromosome 5HL telomere revealed a single gene promoting dormancy during seed development in a 0.7 cM region between MWG851D and MWG851B and the possibility of a second gene proximal to this interval that reduces dormancy during after-ripening (Gao et al. 2003). It is likely the actual number of genes is greater than the number of QTLs identified and that pleiotropy, epistasis, and clusters of linked genes all play roles in the expression of both PHS and dormancy and the relationship between the two traits.

This study adds to the growing body of knowledge about the inheritance of PHS and dormancy and the relationship between these two somewhat opposite traits in barley and cereals in general. Besides gaining a better understanding of these traits and their relationships, the identification of markers that are linked to chromosome regions that affect these traits presents opportunities for marker-assisted selection, in further genetic study, and in breeding improved cultivars. Extremes of both PHS and dormancy can be detrimental to crop production and end use quality of barley and other cereals. A moderate level of dormancy and/or PHS resistance is desirable in a cultivar. The choice of QTLs or genes is expanding to accomplish a desirable balance between PHS and dormancy. From experience thus far, the chromosome 5HL telomere QTL with a moderate effect up to 21% on PHS and up to 18% on dormancy may be the

best choice for six-row barley. This QTL has consistently expressed over genotypes and environments. However, in two-row barley germplasm with the Harrington background that carries the sprout susceptible allele, this region also affects a number of positive malting quality QTL alleles, which may make it difficult to maintain or improve malting quality with reduced PHS potential (Li et al. 2003). Other QTLs that show potential, but probably require more study are the chromosome 3HC ABG399A–DD1.1B QTL and the chromosome 5HL WG908–ABG495A QTL that showed up to 15 and 19% effects on PHS, respectively, and up to 10 and 6% effects on dormancy, respectively.

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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
- Depauw RM, McCaig TN (1991) Components of variation, heritabilities and correlations of indices of sprouting tolerance and seed dormancy in *Triticum* spp. *Euphytica* 52:221–229
- Gao W, Clancy JA, Han F, Jones BL, Budde A, Wesenberg DM, Kleinhofs A, Ullrich SE, NABGP (2004) Fine mapping of a malting quality QTL complex near the chromosome 4H S telomere in barley. *Theor Appl Genet* 109:750–760
- 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, Clancy JA, Jones BL, Wesenberg DM, Kleinhofs A, Ullrich SE (2004) Dissection of a malting quality QTL region on chromosome 1 (7H) of barley. *Mol Breed* 14:339–347

- Han F, Ullrich SE (1994) Mapping of quantitative trait loci for malting quality traits in barley. *Barley Genet Newsl* 23:84–97
- Han F, Ullrich SE, Clancy JA, Jitkov V, Kilian A, Romagosa I (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
- Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Franckowiak J, Rasmusson D, Sorrells M, Ullrich SE, Wesenberg D, Kleinhofs A (1993) Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. *Theor Appl Genet* 87:392–401
- 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
- Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes PM, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. *Theor Appl Genet* 86:705–712
- 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, 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
- Li CD, Tarr A, Lance RCM, Harasymow S, Uhlmann J, Westcot S, Young KJ, Grime CR, Cakir M, Broughton S, Appels R (2003) A major QTL controlling seed dormancy and pre-harvest sprouting/grain α -amylase in two-rowed barley (*Hordeum vulgare*, L). *Aust J Agric Res* 54:1303–1313
- Manly KF, Cudmore RH Jr, Meer JM (2001) Map Manager QTX, cross platform software for genetic mapping. *Mamm Genome* 12:930–932
- 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
- Miura H, Sato N, Kato K, Amano Y (2002) Detection of chromosomes carrying genes for seed dormancy of wheat using the backcross reciprocal monosomic method. *Plant Breed* 121:394–399
- Oberthur L, Blake TK, Dyer WE, Ullrich SE (1995) Genetic analysis of seed dormancy in barley (*Hordeum vulgare* L.). *J Quant Trait Loci*. Available on line at: <http://probe.nalusda.gov.8000/otherdocs/jqtl/jqtl1995-05/dormancy.html>
- 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
- Schwarz P, Henson C, Horsley R (2004) Preharvest sprouting in the 2002 midwestern barley crop: occurrence and assessment of methodology. *J Amer Soc Brew Chem* 62:147–154
- Takeda K (1996) Varietal variation and inheritance of seed dormancy in barley. In: Noda K, Mares DJ (eds) Preharvest sprouting in cereals 1995. Center for Academic Societies, Osaka, Japan, pp 205–212
- 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 Newsl* 45:39–41. <http://wheat.pw.sda.gov/ggpages/BarleyNewsletter/45/Proceedings1.html>
- Ullrich SE, Hayes PM, Dyer WE, Blake TK, Clancy JA (1993) Quantitative trait locus analysis of seed dormancy in “Steptoe” barley. In: Walker-Simmons MK, Reid JL (eds) Preharvest sprouting in cereals 1992. Amer Assoc Cereal Chemist, St. Paul, pp 136–145
- Wan JM, Cao YL, Wang CM, Ikehashi H (2005) Quantitative trait loci associated with seed dormancy in rice. *Crop Sci* 45:712–716
- 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