Theor Appl Genet (2004) 109:62–70
DOI 10.1007/s00122-004-1608-x

ORIGINAL PAPER

D. Prada · S. E. Ullrich · J. L. Molina-Cano · L. Cistué · J. A. Clancy · I. Romagosa

Genetic control of dormancy in a Triumph/Morex cross in barley

Received: 8 August 2003 / Accepted: 19 January 2004 / Published online: 27 February 2004

Abstract Seed dormancy in barley (*Hordeum vulgare* L.) is one of the most important parameters affecting malting. Seed dormancy is quantitatively inherited and variously influenced by the environment. The objectives of the present study were to determine the genome location and effects of quantitative trait loci (QTLs) involved in the expression of seed dormancy in a barley cross between two varieties derived from different germplasm pools. Using a doubled-haploid population of 107 lines of the cross between the malting types Triumph (two-row, dormant) and Morex (six-row, non-dormant), seed dormancy phenotypic data sets from five environments and a 147-marker linkage map were developed in order to perform QTL analyses with simple interval mapping and simplified composite interval mapping procedures. Two different types of variables were considered for seed dormancy characterization: (1) level of dormancy induced during seed development, which was indirectly measured as germination percentage at 3 days and 7 days, GP3 and GP7 respectively; (2) rate of dormancy release in the course of a period after seed harvest (after-ripening). Different mechanisms of genetic control were detected for these two types of dormancy-related traits. A major and consistent dormancy QTL near the centromere on chromosome 7(5H) was associated with the establishment of dormancy during seed development and accounted for 52% and 33% of the variability for GP3 and GP7, respectively. Two other QTLs located in the vicinity of the *vrs1* locus on chromosome 2(2H) and near the long arm telomere on chromosome 7(5H) explained 9% and 19% of variation, respectively, for the rate of dormancy release during after-ripening. Likewise, seed dormancy was assessed in an F_2 population derived from the cross between two dormant types of distinct germplasm groups, Triumph (European, two-row, malt) and Steptoe (North American, six-row, feed), which showed similar but not identical genetic control for dormancy. Interestingly, there is remarkable dormancy QTL conservation in both regions on chromosome 7(5H) identified in this study and among other barley mapping populations. These widely conserved QTLs show potential as targets for selection of a moderate level of seed dormancy in breeding programs.

Communicated by P. Langridge

D. Prada · J. L. Molina-Cano · I. Romagosa (✉)
Centre UdL-IRTA,
Av. Alcalde Rovira Roure 191, 25198 Lleida, Spain
e-mail: iromagosa@pvcf.udl.es
Tel.: +34-973-702534
Fax: +34-973-238264

S. E. Ullrich · J. A. Clancy
Department of Crop and Soil Sciences,
Washington State University,
Pullman, WA 99164-6420, USA

L. Cistué
Estación Experimental de Aula Dei,
CSIC, P.O. Box 202, 50080 Zaragoza, Spain

Introduction

Seed dormancy is defined as the inability of a viable seed to germinate under adequate conditions for germination (Simpson 1990). Moderate seed dormancy in barley (*Hordeum vulgare* L.) is a very important target for many breeding programs when seed is intended for the malting industry. Since seed in the malthouse must germinate rapidly and completely upon imbibition, a high level of dormancy after harvest can become a severe economic problem for maltsters due to storage requirements prior to seed processing. Nevertheless, stringent phenotypic selection against seed dormancy can lead to the development of barley varieties susceptible to germination while still on the mother plant (pre-harvest sprouting), which is also highly undesirable. A balance between a limited level of dormancy at grain maturity and rapid germination after seed harvest is, therefore, highly warranted. However breeding for this balance with phenotypic selection is very difficult as the effects of genotype, environment and genotype × environment (G×E) interaction on seed dormancy are considerable (King 1989).

Genetic variation for dormancy in nature has been described mostly in terms of quantitative inheritance (reviewed in Foley 2001). A classical genetic study revealed that seed dormancy in barley was governed by several recessive loci with high heritability (Buraas and Skinnes 1984). The advent of quantitative trait locus (QTL) analysis provided a powerful tool to dissect complex traits into their genetic components. QTL analyses of barley seed dormancy have been extensively performed in the North American Barley Genome Project (NABGP) double haploid mapping population of Steptoe (dormant)/Morex (non-dormant) cross, and as many as 27 QTLs were detected in one or more environments in the initial study (Ullrich et al. 1993). Major dormancy QTL regions attributed to Steptoe were verified near the centromere and long arm telomere on chromosome 7(5H) and minor QTLs on chromosomes 1(7H) and 4(4H). These were designated, respectively, SD1–SD4 and accounted for 50%, 15%, 5% and 5% of the phenotypic differences in seed dormancy (Han et al. 1996). Based on a detailed study from anthesis through grain-filling, maturity and after-ripening, these four QTLs were observed to have an important effect for dormancy release during after-ripening (Romagosa et al. 1999). In the Harrington (non-dormant)/TR306 (dormant) population of the NABGP, a major dormancy QTL from TR306 accounting for 58% of the phenotypic variation was identified near the long-arm telomere on chromosome 7(5H) as well as a minor QTL (9% variation) from Harrington near the centromeric region of chromosome 5(1H) (Takeda 1996; Ullrich et al. 2002). A study of QTLs controlling two seed germination characters, germination energy and germination capacity, in a cross between the two European spring barley varieties, Blenheim and E224/3, identified several minor QTLs, but few of them were revealed in more than one environment (Thomas et al. 1996).

In the investigation reported here, dormancy QTL analyses were performed in a barley doubled haploid line (DHL) population derived from the cross between Triumph, a major two-row malting cultivar in Europe that is very prone to dormancy, and Morex, a six-row, non-dormant malting-quality standard cultivar in North America. Our objectives were (1) to identify the chromosomal regions involved in the expression of seed dormancy within this population derived from two elite malting standard varieties comprising very distinct barley germplasm groups; (2) to determine the level of conservation of dormancy QTL regions among different populations, particularly with respect to Steptoe/Morex which could be considered as the reference for QTL analysis.

Materials and methods

Plant material

A total of 107 DHLs were produced by anther culture from the F_1 of the cross between Triumph (T; 2-rowed European malting standard barley cultivar with a high level of dormancy) and Morex (M; 6-rowed non-dormant North American malting standard cultivar) at Estación Experimental of Aula Dei, Zaragoza (Spain). The DHLs and the parents were grown in three field environments—Lleida, Spain, 2001, and Pullman, Washington, USA, 2000 and 2001—and under greenhouse and growth room conditions at Lleida and Pullman, respectively, in the spring of 2001.

Additionally, 112 F_2 plants derived from the cross between Triumph and Steptoe (S; 6-rowed North American feed cultivar with high level of dormancy) were developed to relate the genetic control of seed dormancy for the T/M and T/S populations. The T/S F_2 plants and parents were grown in the greenhouse at Lleida in the spring of 2000.

Assessment of seed dormancy

Two different groups of variables were considered for seed dormancy characterization in the T/M DHL population. The first group was determined by the level of seed dormancy at early stages after-harvest, which has been previously induced when the seed is still on the spike of the mother plant. The second group of dormancy-related traits was determined by the rate of dormancy release from the harvest time until the seed reaches a completely non-dormant state.

In order to perform germination tests that determine the dormancy-related variables mentioned above, spikes of the parents and DHLs grown in each environment were harvested at physiological maturity, which was estimated to be the point when all green color is lost from the spike, and hand threshed prior to being placed in storage at –20°C to preserve dormancy. After removal from the freezer, seed was permitted to after-ripen at ambient temperature for 1, 7, 15 and 30 days post-harvest (DPH) prior to conducting germination analysis. Germination tests were carried out in 90-mm petri dishes on Whatman no. 1 filter paper saturated with water. Three replications of 50 seeds at each after-ripening date were germinated at 20°C in the dark.

North American and European researchers have mostly followed different protocols to determine germination percentage as an indirect measurement for seed dormancy. The NABGP, following the standard germination protocol of the Association of Official Seed Analysis (AOSA 1988), estimates seed dormancy as the percentage of germinated seeds after 7 days of incubation, while in Europe, seed dormancy has been mostly determined by the percentage of germinated seeds after 3 days, as recommended by the Analytica of the European Brewery Convention (EBC 1987). In order to detect whether these variables affecting seed dormancy aspects are under the same or different genetic control, we counted germinated seeds (coleoptile emerged through the hull) at both 3 days and 7 days after incubation and expressed the results as a percentage of the total. Hereafter, germination percentages at 3 days and 7 days are referred to as GP3 and GP7, respectively. The level of dormancy induced through seed development was estimated for GP3 and GP7 at an early stage after harvest (7 DPH) and designated as $GP3_{(7)}$ and $GP7_{(7)}$.

The rate of dormancy release through after-ripening was statistically characterized by simple linear regression analysis with the slope of the straight-line with GP7 as the dependent variable and days post-harvest, ranging from 1 DPH to 30 DPH, as the independent variable. For the determination of these slopes, occasional GP7 values over 98% at 7 DPH were not used because they represent samples that were non-dormant. $GP3_{(7)}$ and $GP7_{(7)}$, jointly with the slopes of straight lines of dormancy release, were independently estimated for the parents and DHLs from the five environments used.

Likewise, the level of seed dormancy in the F_2 population derived from the Triumph/Steptoe (T/S) cross was estimated by GP3 at 25 DPH [$GP3_{(25)}$] following the germination protocol described above (EBC 1987).

Molecular and morphological marker analyses

The DHLs were scored for a range of molecular and morphological markers to produce a genetic map. Five morphological traits were determined and designated with the nomenclature proposed by Franckowiack (1997): (1) *Lga* = long glume awn, (2) *Gth* = toothed lemma, (3) *vrs1* = six-rowed spike, (4) *int-c* = intermedium spike, (5) *srh* = short rachilla hair. For molecular marker analyses, genomic DNA was extracted from the leaves of greenhouse-grown seedlings of the entries using a CTAB extraction method (Saghai-Maroof et al. 1984). Simple sequence repeat (SSR) primer sequences were obtained and analyses were performed according to the protocols of Becker and Heun (1995) and Ramsay et al. (2000). Amplified fragment length polymorphism (AFLP) analyses were conducted following the procedure of Vos et al. (1995). Twelve *Eco*RI+3/*Mse*I+3 AFLP primer combinations were analyzed, and the polymorphic markers obtained were designed with the nomenclature proposed by Qi and Lindhout (1997). Ten sequence-tagged site (STS) markers distributed on the seven barley chromosomes were analyzed according to Sayed-Tabatabaei et al. (1999), who also published the sequences.

Linkage map construction

Linkage analysis of the molecular and morphological trait data was performed with MAPMAKER 3.0 (Lander et al. 1987) using the Kosambi mapping function (Kosambi 1944). Morphological, STS and SSR markers with common chromosomal locations in previously published maps (Kleinhofs et al. 1993; Ramsay et al. 2000) were used as anchor markers to assign linkage groups to chromosomes. Markers showing severe distortion were dropped for linkage analysis. Two- and three-point analyses were conducted at LOD 3.0, and markers were assigned to linkage groups defined by anchor markers using the "assign" command. The "order" command (LOD 3.0) was used to order markers within linkage groups, and those markers without unique placement were integrated by the "build" command. Following these procedures, all markers uniquely placed were included in the genetic map.

Statistical analyses

QTL analyses were performed with the software package MQTL (Tinker and Mather 1995) adapted for the evaluation progeny in multiple environments to identify possible QTL × environment (E) interactions. Genome-wide QTL searches were performed by both simple interval mapping (SIM) and simplified composite interval mapping (sCIM), each with a test statistic for QTL main effect and for QTL×E interaction across sites. Significance thresholds for the SIM test statistic to maintain the genome-wise Type I error rate below 5% were established by using 5,000 random permutations of the data. For sCIM analysis, 27 background markers, of which 24 were well scattered along the genome and three located in regions where there was evidence for a QTL based on SIM analysis, were selected as cofactors to control the effect of the genetic background. Significant QTLs were declared at positions where both SIM and sCIM peaks were coincident, SIM peaks exceeded the significance thresholds ($P \leq 0.05$) and sCIM peaks were also strong. This is in accordance with the nomenclature of *primary* QTL proposed by Tinker et al. (1996). QTL position corresponded to the peaks of sCIM scans. Individual and joint additive effects of QTLs were used to estimate the percentage of phenotypic variation (R^2p and mR^2p) accounted for by significant QTLs and confirmed by stepwise multi-locus linear models using SAS procedures (SAS Institute 2001).

Pearson's correlation coefficients were calculated between dormancy traits. For each trait, estimates of heritability were based on the means of replicated testing within environments and calculated as: $h^2=\sigma^2_g/(\sigma^2_g+\sigma^2_e/e)$; where σ^2_g is the variance among DHLs, σ^2_e is the error variance among DHLs and e is the number of environments. All statistical test procedures were performed using SAS software (SAS Institute 2001).

Results and discussion

Dormancy in parents and DHL population

Continuous distribution frequencies for the percentage of germinated seeds after 3 days and 7 days of incubation determined at 7 DPH [$GP3_{(7)}$ and $GP7_{(7)}$, respectively] and for the rate of dormancy release through after-ripening (DR) were observed for the T/M population for each environmental condition (Fig. 1). Morex consistently had higher $GP3_{(7)}$ and $GP7_{(7)}$ than Triumph in all environments. Both variables ranged from low values, which reflect almost full dormancy, to values of 100% germination, indicating no dormancy. Although positive

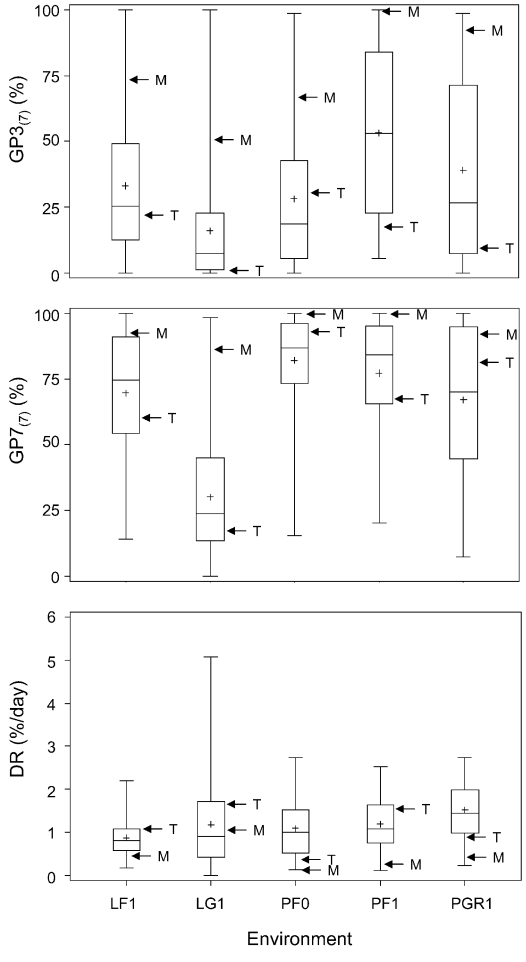


Fig. 1 Means of Triumph (*T*), Morex (*M*) and their DH progeny (+, *cross*) for the percentage of germinated seeds after 3 days and 7 days of incubation at 7-days post-harvest [$GP3_{(7)}$ and $GP7_{(7)}$, respectively] and for dormancy release (*DR*), together with ranges, 25–75% quartile (*box*), and median (*horizontal line*) across five environments. Environments are coded as follows: *L* Lleida, *P* Pullman, *F* field, *G* greenhouse, *GR* growth room, *0* year 2000, *1* year 2001

Table 1 Pearson phenotypic correlation coefficients of the barley Triumph/Morex DH progenies grown in the five environments used for the percentage of germinated seeds after 3 days and 7 days of incubation at 7-days post-harvest [$GP3_{(7)}$ and $GP7_{(7)}$, respectively] and dormancy release (DR)

Environment[a]	Correlation coeficients[b]		
	$GP3_{(7)}$/$GP7_{(7)}$	$GP3_{(7)}$/DR	$GP7_{(7)}$/DR
LF1	0.77**	−0.37**	−0.65**
LG1	0.86**	0.06NS	−0.12NS
PF0	0.61**	−0.61**	−0.98**
PF1	0.78**	−0.73**	−0.94**
PGR1	0.66**	−0.72**	−0.96**
Overall	0.77**	−0.47**	−0.75**

[a] Environments are coded as follows: L, Lleida; P, Pullman; F, field; G, greenhouse; GR, growth room; 0, year 2000; 1, year 2001
[b] Correlation coefficients are significant at: $^{**}P \leq 0.01$. NS, Not significant

and negative transgressive segregants for $GP3_{(7)}$ and $GP7_{(7)}$ were observed, the number of DHLs with a lower $GP7_{(7)}$ value than Triumph had the greatest importance. In general, the average of the DHLs for $GP3_{(7)}$ and $GP7_{(7)}$ was significantly lower than the mid-parent value and tended to be skewed toward the Triumph values from each of the five environments. In some cases, the average of the DHL population for $GP7_{(7)}$ was even lower than Triumph—for example, in Pullman under field conditions in 2000 (PF0) and in Pullman in the growth room in 2001 (PGR1). These results indicate a strong contribution of both parents to positive and negative alleles in the expression of the two traits.

Dormancy release is a progressive process from time of maturity, when the level of seed dormancy previously imposed during seed development dissipates, until a complete non-dormant state, which is achieved at the end of the after-ripening period. The rate of dormancy release (DR) during after-ripening is defined as the simple linear regression with the slope of the straight-line with GP7 as the independent variable and days post-harvest as the dependent variable. Therefore, a sharper increase in the slope of DR is expected for Triumph due to its lower GP7 value at the beginning of after-ripening with respect to Morex. In fact, Triumph consistently showed higher DR than Morex in all of the five environments tested (Fig. 1). DR rates ranged between 0 and 3% per day in all environments except in Lleida under greenhouse conditions (LG1), where it appears that under these controlled growing conditions dormancy was highly induced throughout grain development because average $GP3_{(7)}$ and $GP7_{(7)}$ were skewed to low values. Therefore, the range of DR was much wider in LG1 than in the rest of the environments.

In order to estimate the relationships among the three dormancy-related traits, we calculated the correlation coefficients between them at each environment (Table 1). For seed dormancy induction, $GP3_{(7)}$ and $GP7_{(7)}$ were strongly and positively correlated under all environments with an overall correlation coefficient of 0.77. Conversely, highly significant negative correlations were observed between DR and both $GP3_{(7)}$ and $GP7_{(7)}$ in all environments except in LG1, with overall correlations of −0.47 and −0.75, respectively.

Construction of the genetic map

The Triumph/Morex map constructed includes 45 SSR, five morphological, two STS and 95 AFLP markers, and has a total length of 1,125 cM with an average marker spacing of 7.5 cM (Fig. 2). By using 12 primer combinations, 122 polymorphic AFLP marker loci were observed (average of 10.1 per primer combination), but 27 (22%) of them were dropped due to missing data, lack of linkage detection or non-unique placements. Only two STSs (MWG2033 and MWG522) out of the ten initially screened were polymorphic between Triumph and Morex. Among 55 polymorphic SSR markers, 10 (18%) were severely distorted and not included in the map. However, three regions of segregation distortion ($P \leq 0.05$), located on chromosomes 1(7H), 3(3H) and 7(5H), were observed. The regions on chromosome 1(7H) and 7(5H) were skewed towards the Triumph allele and the one on chromosome 3(3H) towards the Morex allele.

The clustering of AFLP markers was observed in centromeric regions. This phenomenon has been reported in several other barley mapping populations with this type of markers (Costa et al. 2001). Slight differences in the order and estimated distances between markers from previously published barley maps were detected. In all cases the linear order of the SSRs corresponds to published data of Ramsay et al. (2000) except on chromosome 3(3H) where an inversion from the expected order was detected between HVM70 and HVM62 (Fig. 2). The STS placements are in complete accordance with the location of the corresponding RFLP markers published by Kleinhofs et al. (1993).

Detection of dormancy QTLs

From the initial 147-point map, we developed a skeleton linkage map which we used this for mapping dormancy QTLs by eliminating co-segregating markers and those linked closer than 1 cM. Significant QTL main effect and QTL×E interaction were found for all three dormancy-related characters (Fig. 3, Table 2).

One major $GP3_{(7)}$-QTL was detected ($P \leq 0.05$) near the centromere on chromosome 7(5H) in the E32M49h-E32M49e interval (Fig. 3A, Table 2). The allele for high dormancy attributed to Triumph had a large (52% of phenotypic variation) and consistent effect expressed in all five environments. Based on the 86% heritability (h^2) estimate for this trait, a high portion of the genotypic variance was accounted for by this single QTL. A highly significant QTL×E interaction (non-crossover) site was coincident with the QTL main effect peak. This interaction occurred because of the large differences in magni-

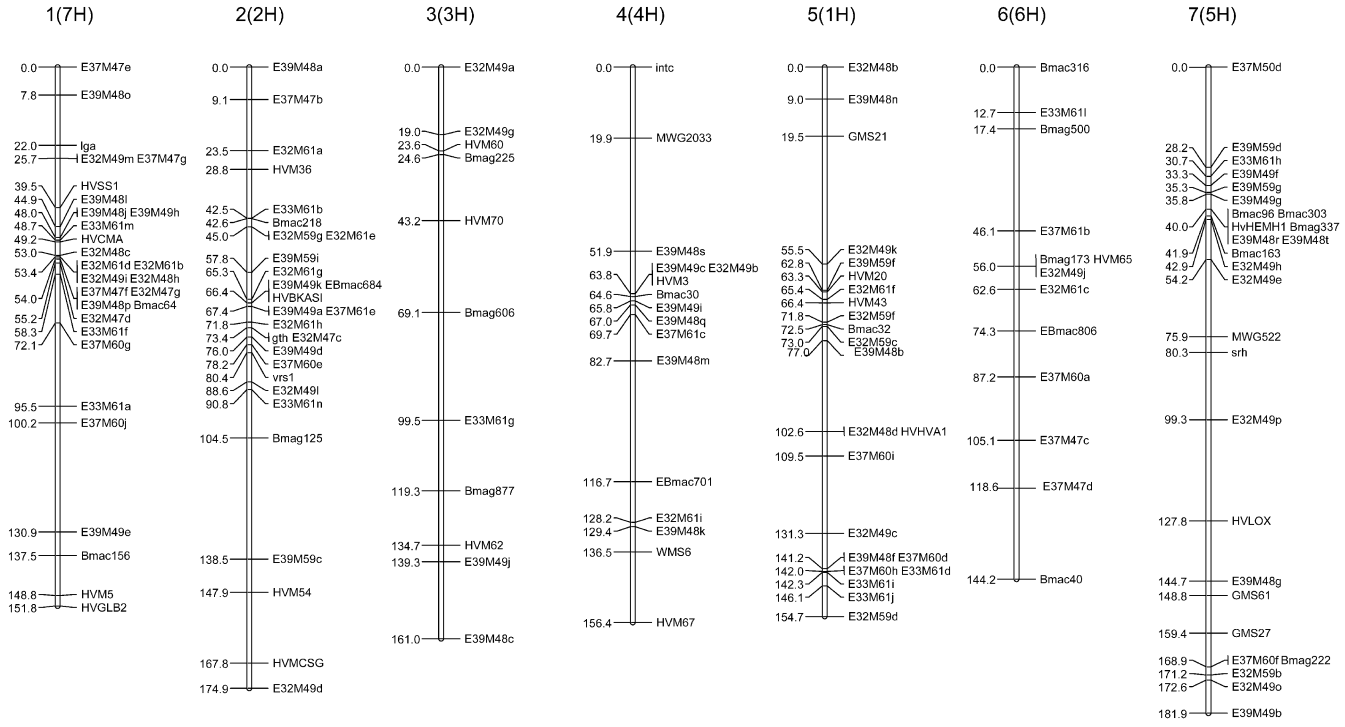


Fig. 2 Linkage map based on a mapping population of 107 DH lines derived from the Triumph/Morex barley cross. Chromosomes are oriented with short arms at the *top* of the figure. Map distances, given on the *left side* of the *bars*, are in centiMorgans

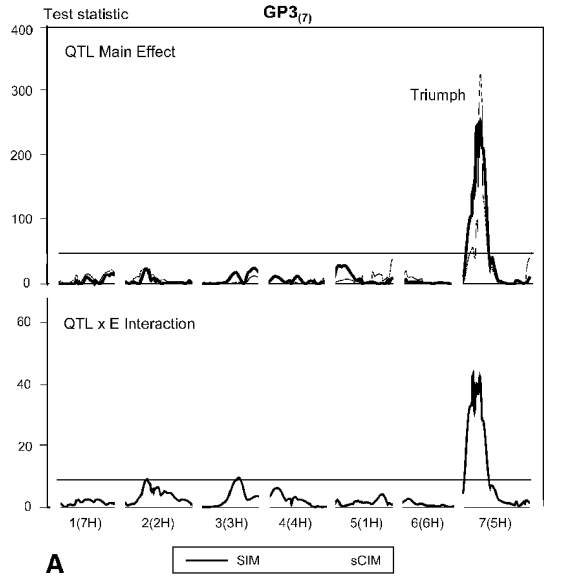
Table 2 QTL main effects in the barley Triumph/Morex DH progeny across five environments

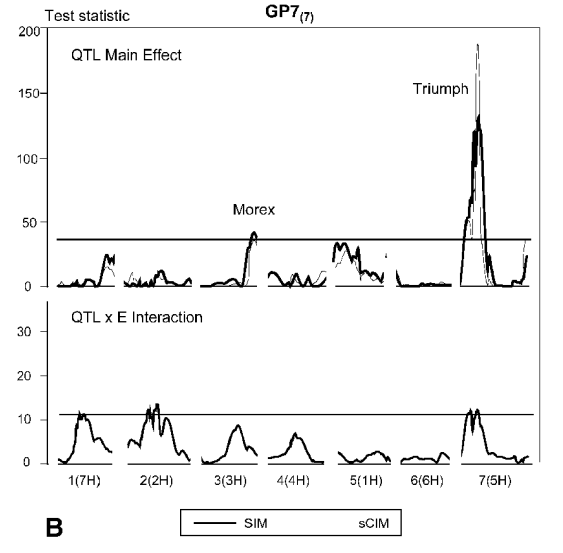
Dormancy variables[a]	Chromosome	Marker interval	Position[b] (cM)	Test statistic[c]		LOD[d]	Allelic effect[e]	Variance explained[f] (%)	
				SIM	sCIM			R^2p	mR^2p
$GP3_{(7)}$	7(5H)	E32M49 h–E32M49e	46.5	252	326	15.4	42 (T)	52	52
$GP7_{(7)}$	7(5H)	E32M49 h–E32M49e	48.5	131	181	8.5	25 (T)	33	40
	3(3H)	E39M49j–E39M48c	152.3	42	36	3	15 (M)	13	
DR	7(5H)	E39M49b	179.3	22	34	4	0.31 (T)	19	26
	2(2H)	*vrs1*	82.3	14	24	2.3	0.24 (T)	9	

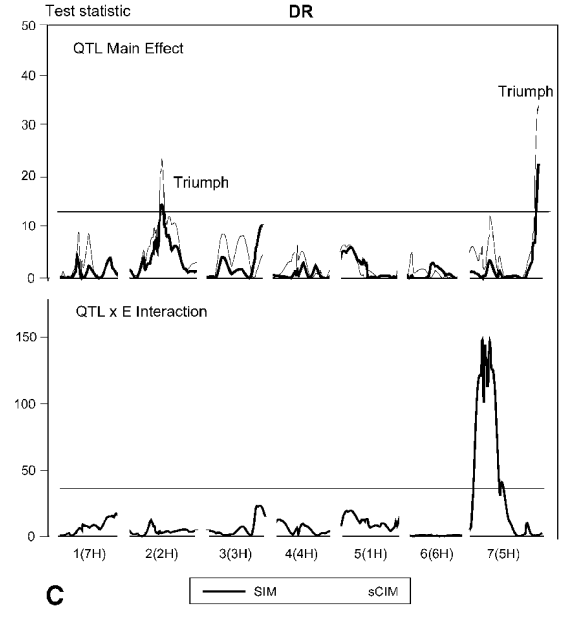
[a] Dormancy variables: $GP3_{(7)}$, percentage of germinated seeds after 3 days of incubation at 7 days post-harvest (DPH); $GP7_{(7)}$, percentage of germinated seeds after 7 days of incubation at 7 DPH; DR, dormancy release through after-ripening
[b] QTL position determined by simplified composite interval mapping (sCIM) peak
[c] Test statistic with MQTL (Tinker and Mather 1995). Threshold significance is at $P \leq 0.05$. Only QTLs with coincident peaks for simple interval mapping (SIM) and sCIM analyses are included
[d] According to Tinker and Mather (1995). LOD score is approximately equivalent to the SIM averaged over environments divided by $2 \times \ln(10)$
[e] Dormant effect attributed to: M, Morex; T, Triumph
[f] Individual (R^2p) and multi-locus (mR^2p) percentage of variance explained by each QTL

tude of the QTL main effect among the five environments. In all cases Triumph was the source of the dormant allele. Although two additional regions on chromosomes 2(2H) and 3(3H) showed significant QTL×E interaction ($P \leq 0.05$) for $GP3_{(7)}$, the effects were small according to the SIM-significance threshold (Fig. 3A).

For $GP7_{(7)}$, primary QTL regions were located near the centromere on chromosome 7(5H) and long-arm telomere on chromosome 3(3H) (Fig. 3B, Table 2). The dormant allele on chromosome 7(5H) was attributed to Triumph (33% of phenotypic variation) and was expressed in all five test environments. In contrast, the dormant allele on chromosome 3(3H) was attributed to Morex (13% of phenotypic variation) and was only expressed in two environments—LF1 and PF0. The detection of this QTL with an allelic effect opposite to that predicted by parental performance is suggested by the large number of negative transgressive lines with $GP7_{(7)}$ values lower than Triumph, particularly in LF1 and PF0 (Fig. 1). It is most likely that, since the parents are genetically distinct malt standard varieties, positive and negative transgressive segregants would be predicted with alleles from both parents contributing to seed dormancy. The detection of QTLs with allelic effects opposite to those predicted by the phenotype of a given parent has been described in many barley populations for complex traits. The cause







most often proposed for transgression is the accumulation in certain progeny of complementary alleles at multiple loci inherited from both parents of a cross (Tanksley 1993). The additive effect of these two significant $GP7_{(7)}$-QTLs explained 40% of the phenotypic variation (Table 2). When the effect of the secondary QTL placed near the long-arm telomere of the chromosome 7(5H) (with only a sCIM peak) (Fig. 3B) was included in the model, 51% of the total phenotypic variation was accounted for. Considering that the h^2 estimate for $GP7_{(7)}$ was 84%, a large proportion of genotypic variance is explained by the combined model. Overall, there was little QTL×E interaction for this trait, and although three regions of the barley genome on chromosomes 1(7H), 2(2H) and 7(5H) were detected ($P \leq 0.05$), in all cases the QTL×E effect was relatively small based on the SIM significance threshold (Fig. 3B).

The placement of the single major $GP3_{(7)}$-QTL in the centromeric region on chromosome 7(5H) is coincident with the major $GP7_{(7)}$-QTL (Fig. 3A, B, Table 2). These QTLs were found in a region that partially showed a given degree of distortion towards the Triumph allele. However, this distortion was not extreme (68 lines carrying the Triumph allele versus 39 carrying the Morex allele), and significant dormancy effects were clearly detected for close-by markers that did not show distortion. Therefore, although the true estimate (both in terms of effect and position) may be slightly biased, the association of this region in chromosome 7(5H) with dormancy is clearly established. The $GP3_{(7)}$ and $GP7_{(7)}$ values had a strong positive correlation in all of the five environments used (Table 1). These data have important practical implications because a similar genetic control was detected when the dormancy of $GP3_{(7)}$ was evaluated with the EBC germination protocol (EBC 1987) extensively used in Europe and that of $GP7_{(7)}$ with the AOSA procedure (AOSA 1988) and followed by North American researchers. Whether this common QTL position for the two dormancy-related traits is due to linkage or pleiotropy cannot be determined given the level of resolution afforded within the T/M mapping population reported here. However, fine mapping efforts on this region are currently underway to estimate the genetic relationships between both traits more precisely. A marker-assisted backcross procedure is being used to develop sets of near-isogenic lines with Triumph chromosome segments at this QTL region in a Morex genetic background.

Fig. 3A–C Scans of tests for simple interval mapping (SIM, *wide line*) and simplified composite interval mapping (sCIM, *fine line*) for QTL main effects and QTL × environment (*E*) interaction of the dormancy-related traits. **A** Percentage of germinated seeds after 3 days of incubation at 7-days post-harvest [$GP3_{(7)}$], **B** percentage of germinated seeds after 7 days of incubation at 7-days post-harvest [$GP7_{(7)}$], **C** rate of dormancy release throughout the after-ripening period (*DR*). Barley chromosomes are shown in order, *left to right*, oriented with the short arm on the *left*. *Horizontal lines* show thresholds for SIM estimated from 5,000 permutations. The parent giving the dormant effect allele is shown for each QTL peak

During after-ripening, two QTL regions involved in DR were located near the long-arm telomere on chromosome 7(5H) (19% of phenotypic variation) and in chromosome 2(2H) (9% variation) (Fig. 3C, Table 2). In both cases Morex (non-dormant parent) contributed the higher rate of DR. The two-locus interaction (epistasis) between these QTLs for DR was not significant ($P \leq 0.01$). The h^2 estimate for DR was 88%, and although no significant QTL×E interaction was detected at either of the QTL sites, a high quantitative interaction was observed where the major QTLs for $GP3_{(7)}$ and $GP7_{(7)}$ were located on chromosome 7(5H) (Fig. 3C). The strong and negative correlation detected for DR with respect to $GP3_{(7)}$ and $GP7_{(7)}$ (Table 1), jointly with the presence of a significant QTL×E peak for DR where the major $GP3_{(7)}$- and $GP7_{(7)}$-QTL main effect was placed (Fig. 3C), emphasize the relationship between induction of dormancy in the course of the seed development and recovery from the dormant state during after-ripening.

Interestingly, the DR-QTL on chromosome 2(2H) is coincident with the *vrs1* locus. The *vrs1* locus, together with the *int-c* locus on the short arm of chromosome 4(4H), controls the two-/six-rowed inflorescence type (Franckowiack 1997). The *vrs1* locus has been shown to have a large effect on several malting-quality traits (Marquez-Cedillo et al. 2000). In fact, within the T/M mapping population, QTL analyses for thousand-kernel weight and test weight, conducted at Lleida under field conditions in 2002 (data not shown), confirmed the importance exerted by the *vrs1* locus in the expression of both kernel quality traits, explaining 71% and 53% of the phenotypic variance, respectively. It is most likely that the effect of *vrs1* on DR during after-ripening could be at least partly associated with the physical differences of the kernels displayed by the two-rowed and six-rowed types. This is consistent with the idea that the higher uniform grain of two-rowed types germinates more uniformly than that of six-rowed types (Ayoub et al. 2002). Accordingly, differences in shape and size of the kernel between the two-rowed and six-rowed types could presumably be responsible for differences in water uptake capacity, which is required before triggering of the germination process.

In conclusion, dormancy analysis in this study provided not only genome locations of the regions affecting the trait, but insights into the differential expression of those regions depending upon the seed developmental stage. The dormant allele attributed to Triumph for the QTL in the centromeric region on chromosome 7(5H) has a strong effect on the physiological pathway leading to the imposition and maintenance of dormancy in developing grain. On the contrary, the physiological mechanisms of recovery from dormancy after harvest remain largely unknown, but the genetic control is mainly regulated by the dormancy-release allele of Morex in the QTL near the long-arm telomere on chromosome 7(5H). A moderate level of dormancy could be achieved, therefore, by manipulating the balance between these two loci. Maintaining the Triumph dormancy allele at the centromeric QTL could prevent pre-harvest sprouting, while introducing the Morex dormancy-release allele at the telomere could lead to a rapid and uniform germination after harvest. Both of these situations are highly desirable when seed is intended for malting.

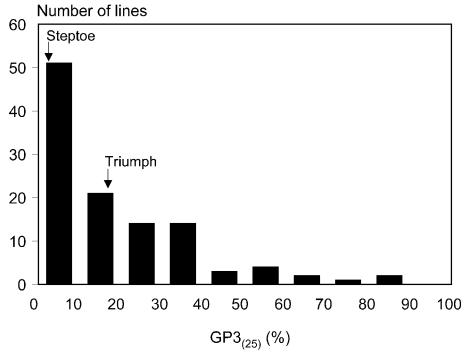


Fig. 4 Frequency distribution for the percentage of germinated seeds after 3 days of incubation at 25-days post-harvest [$GP3_{(25)}$] for the Triumph/Steptoe F_2 plants and parents under greenhouse growing conditions in Lleida, 2000

Conservation of dormancy QTLs among crosses

Several studies have identified the presence of two major dormancy QTLs in the Steptoe/Morex (S/M) cross located in the centromeric region (SD1) and long-arm telomere on chromosome 7(5H) (SD2) (Ullrich et al. 1993; Oberthur et al. 1995; Han et al. 1996). In addition, a major dormancy QTL (58% of the variation) in the Harrington/TR306 (H/T) DHL population (Takeda 1996; Ullrich et al. 2002) and in the Chebec/Harrington (C/H) mapping population (Karakousis et al. 1996) have been identified on the long-arm telomere on chromosome 7(5H).

Interestingly, in the Triumph/Morex (T/M) population reported herein, two dormancy QTLs were placed at coincident positions with respect to S/M QTLs: a major QTL associated with the establishment of dormancy during seed development located at the centromeric region on chromosome 7(5H) (Fig. 3A, B) and a dormancy-release QTL near the long-arm telomere on chromosome 7(5H) (Fig. 3C). The dormancy QTLs detected in the S/M cross, both attributed to Steptoe, explained 50% and 15% of the phenotypic differences in the averaged germination percentage at several post-harvest dates (Han et al. 1996). The two major QTLs detected in the T/M cross could be allelic with SD1 and SD2 found in the S/M cross and with those detected in the H/T and C/H populations.

Since one of the main objectives of the present study was to relate the mechanisms of genetic control in the T/M DHL population with those of the S/M mapping population, which is considered to be the reference for barley QTL analysis, a cross between Triumph and Steptoe was made. GP3 at 25 DPH [$GP3_{(25)}$] was determined on F_2 progeny grown at Lleida in 2000 (Fig. 4). The average $GP3_{(25)}$ for the 112 F_2 T/S plants

was 19±2% (mean ± standard error), with a range of 0–88%. Although Steptoe and Triumph display high levels of dormancy, differences in germination were observed. $GP3_{(25)}$ for Steptoe and Triumph were 2% and 19%, respectively. The distribution of the F_2 population was continuous and highly skewed to low GP3 values, but a large number of positive transgressive segregants were obtained. This level of dormancy induced in the F_2 progeny seems to confirm that although the two parents were derived from very distinct germplasm pools, at least partial common genetic control of dormancy induction is present. Such a mechanism could likely be controlled in both cultivars by the combined effect of the SD1 and SD2 loci on chromosome 7(5H). If Steptoe and Triumph had exactly the same alleles for dormancy, all of the F_2 progeny should express a high level of dormancy [low $GP3_{(25)}$]. Since there were some transgressive progeny with relatively high $GP3_{(25)}$, there appears to be some difference in the operation of the genes controlling dormancy in these two cultivars. The existence of other 'minor' genes seems to be confirmed. In fact, based on the QTL analysis of the S/M DHL population, several minor QTLs were only expressed in some of the environments—for example, SD3 on chromosome 1(7H) and SD4 on chromosome 4(4H) (Ullrich et al. 1993, 1995; Han et al. 1996). In the case of the T/M DHL population reported here, additional minor QTLs were detected under specific environmental conditions in the vicinity of the *vrs1* locus on chromosome 2(2H) for DR and in chromosome 3(3H) L telomere for $GP7_{(7)}$.

Based on the high level of conservation of SD1 and SD2 among barley populations, it is important that fine mapping efforts within the S/M population has resolved SD1 into a 4.4-cM region from a 8.7-cM region (Han et al. 1999) and SD2 into a 0.8-cM region from a 9-cM region (Gao et al. 2003). Both regions are promising candidates for dormancy genes for barley breeders. Further work should be done to gain a better understanding of the regulation of seed dormancy exerted by these two widely conserved QTLs. The results reported herein seem to illustrate that such genomic regions on chromosome 7(5H) are critical to maintaining a particular barley genomic architecture for dormancy and could, therefore, be candidates to construct an ideal barley ideotype for desired levels of dormancy in practical breeding programs.

Acknowledgements The authors thank Vadim Jitkov and Mellissa Dugger for assistance in the field. The financial support of the Comisión Interministerial de Ciencia y Tecnología (Competitive Grants AGF98-0251-C03–01 and AGF99-1024), which funded the Spanish teams, and of the North American Barley Genome Project through USDA-CSREES Special Grant Contract no. C0203A-02, Project 4206 and the Washington State Agricultural Research Center Project 1006 to the USA team are gratefully acknowledged.

References

AOSA (1988) Association of Official Seed Analysis for testing seeds. J Seed Technol 12

Ayoub M, Symons SJ, Edney MJ, Mather DE (2002) QTLs affecting kernel size and shape in a two-rowed by six-rowed barley cross. Theor Appl Genet 105:237–247

Becker J, Heun M (1995) Barley microsatellites: allele variation and mapping. Plant Mol Biol 27:835–845

Buraas T, Skinnes H (1984) Genetic investigations on seed dormancy in barley. Hereditas 101:235–244

Costa JM, Corey A, Hayes PM, Jobet C, Kleinhofs A, Kopisch-Obusch A, Kramer SF, Kudrna D, Li M, Riera-Lizarazu O, Sato K, Szucs P, Toojinda T, Vales MI, Wolfe RI (2001) Molecular mapping of the Oregon Wolfe barleys: a phenotypically polymorphic doubled-haploid population. Theor Appl Genet 103:415–424

EBC (1987) European brewery convention. Analytica EBC, 4th edn. Brauerei und Gertränke Rundschau, Zurich

Foley ME (2001) Seed dormancy: an update on terminology, physiological genetics, and quantitative trait loci regulating germinability. Weed Sci 49:305–317

Franckowiack J (1997) Revised linkage maps for morphological markers in barley (*Hordeum vulgare* L.). Barley Genet Newsl 26:9–21

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 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

Karakousis A, Kretschmer J, Manning S, Chalmers K, Langridge P (1996) The Australian barley genome mapping project. Available on-line at: http://greengenes.cit.cornell.edu/WaiteQTL

King RW (1989) Physiology of sprouting resistance. In: Derera NF (ed) Pre-harvest field sprouting of cereals. CRC Press, New York, pp 27–55

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, Franckowiack JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of barley genome. Theor Appl Genet 86:705–712

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincon SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Marquez-Cedillo LA, Hayes PM, Jones BL, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich SE, Wesenberg DM (2000) NABGMP: QTL analysis of malting quality in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups. Theor Appl Genet 101:173–184

Oberthur L, Blake TK, Dyer WE, Ullrich SE (1995) Genetic analysis of seed dormancy QTL in reciprocal backcross populations. Available on-line at: J Agric Genomics http://www.ncrg.org/jag/papers95/paper596/index595.html

Qi X, Lindhout P (1997) Development of AFLP markers in barley. Mol Gen Genet 254:330–336

Ramsay L, Macaulay M, Degli-Ivanissevich S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

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

Saghai-Maroof MA, Soliman KA, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

SAS Institute (2001) SAS user's guide statistics, ver. 8. SAS Institute, Cary, N.C.

Sayed-Tabatabaei BE, Komatsuda T, Takaiwa F, Graner A (1999) DNA sequencing and primer designing for RFLP clones evenly distributed in the barley genome. Barley Genet Newsl 28:15–18

Simpson GM (1990) Seed dormancy in grasses. Cambridge University Press, New York

Takeda K (1996) Varietal variation and inheritance of seed dormancy in barley. In: Noda K, Mares DJ (eds) Proc 7th Int Symp Pre-harvest Sprouting Cereals. Center for Academic Societies of Japan, Osaka, pp 205–212

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233

Thomas WT, Powell W, Swanston JS, Ellis RP, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Waugh R, Smith DB (1996) Quantitative trait loci for germination and malting quality characters in a spring barley cross. Crop Sci 36:265–273

Tinker NA, Mather DE (1995) MQTL: software for simplified composite interval mapping of QTL in multiple environments. Available on-line at: J Agric Genomics http://www.ncrg.org/jag/papers95/paper295/index295.html

Tinker NA, Mather DE, Rossnagel BG, Kasha KJ, Kleinhofs A, Hayes PM, Falk DE, Ferguson T, Shugar LP, Legge WG, Irvine RB, Choo TM, Briggs KG, Ullrich SE, Franckowiack JD, Blake TK, Graf RJ, Dofing SM, Shagai-Maroof MA, Scoles GJ, Hoffman D, Dahleen LS, Kilian A, Chen F, Biyashev RM, Kudrna DA, Steffenson BJ (1996) Regions of the genome that affect agronomic performance in two-row barley. Crop Sci 36:1053–1062

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) Proc 6th Int Symp Pre-harvest Sprouting Cereals. Am Assoc Cereal Chemist, St Paul, Minn., pp 136–145

Ullrich SE, Han F, Blake TK, Oberthur L, Dyer WE, Clancy JA (1995) Seed dormancy in barley: genetic resolution and relationship to other traits. In: Noda K, Mares DJ (eds) Proc 7th Int Symp Pre-harvest Sprouting Cereals. Center for Academic Societies of Japan, Osaka, pp 157–163

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. Available on-line at: http://wheat.pw.usda.gov/gg-pages/BarleyNewsletter/45/Proceedings1.htm

Vos P, Hogers M, Bleeker M, Reijans M, van de Le T, Hornes M, Frijiters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414