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Molecular dissection of a dormancy QTL region near the chromosome 7 (5H) L telomere in barley

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Abstract Moderate seed dormancy is desirable in barley (*Hordeum vulgare* L.). It is difficult for breeders to manipulate seed dormancy in practical breeding programs because of complex inheritance and large environmental effects. Quantitative trait locus (QTL) mapping opens a way for breeders to manipulate quantitative trait genes. A seed dormancy QTL, SD2, was mapped previously in an 8-cM interval near the chromosome 7 (5H) L telomere from a cross of 'Steptoe' (dormant)/'Morex' (non-dormant) by the North American Barley Genome Project using an interval mapping method and a relatively low-resolution genetic map. SD2 has a moderate dormancy effect, which makes it a promising candidate gene for moderate seed dormancy in barley cultivar development. The fine mapping of SD2 is required for efficient manipulation of SD2 in breeding and would facilitate the study of dormancy in barley. Ten different Morex isolines were generated, including regenerated Morex, of which nine lines had duplicates. The isolines together with Steptoe and Morex were grown in growth room and field environments for 2 years (2000 and 2001). In the growth room, relatively low growing temperatures (25 °C day/15 °C night) were employed to promote seed dormancy development. Seed germination percentage, determined at different post-harvest after-ripening periods, was used to measure seed dormancy. Fine mapping using the substitution mapping method based on differences among isolines resolved the SD2 QTL into an 0.8-cM interval between molecular markers MWG851D and MWG851B near the chromosome 7 (5H) L telomere. Relatively low temperatures ($\leq$25 °C) during seed development promoted the expression of the SD2 dormancy QTL. The chromosome region above the MWG851D-MWG851B interval might play a role in reducing barley seed dormancy during after-ripening.

Keywords *Hordeum vulgare* · QTL · Fine mapping · Dormancy

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The North American Barley Genome Project is considered to be one of the authors.

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Introduction

Seed dormancy is defined as the failure of viable mature seeds to germinate under favorable environmental conditions. As it currently cannot be measured directly, seed germination percentage is usually employed to measure it. Barley seed dormancy is a quantitative trait that is affected by several genes, environmental factors, and by gene × environment interaction (King 1989; Ullrich et al. 1996). Barley production and end-use quality require a certain level of seed dormancy, and the lack of or low seed dormancy can lead to pre-harvest sprouting and low malting quality. High seed dormancy can result in non-uniformity of seed germination causing field stand establishment problems and low malt extract yield (Ullrich et al. 1997). Generally, a moderate level of seed dormancy is thought to be appropriate for barley cultivars (Han et al. 1999; Romagosa et al. 1999).

From the 'Steptoe'/'Morex' cross, which is one of the major barley mapping crosses of the North American Barley Genome Project (NABGP) (Kleinhofs et al. 1993), four seed dormancy quantitative trait loci (QTLs), designated SD1, SD2, SD3 and SD4, have been detected using interval mapping methods (Ullrich et al. 1993;

Oberthur et al. 1995; Han et al. 1996). Steptoe contributes all four dormancy alleles. SD1 and SD2 on chromosome 7 (5H), which were expressed in all of the growing conditions examined with relatively large dormancy effects, accounted for about 50% and 15% of the dormancy variability, respectively; SD3 on chromosome 1 (7H) and SD4 on chromosome 4 (4H) expressed only under certain growing conditions with relatively small effects, each accounting for about 5% of the variability (Han et al. 1996). Although interaction between SD1 and SD2 was detected, the removal of the Steptoe allele of SD1 slightly increased the expression of SD2 (Han et al. 1996). SD2 was located in the 8-cM interval between markers ABC309 and MWG851 on chromosome 7 (5H) L near the telomere (Ullrich et al. 1996).

QTL analysis often only locates QTLs to approximate chromosome regions because of (1) the relatively low resolution of available genetic maps, (2) the limited availability of molecular markers, and (3) the low accuracy of QTL-mapping software. Fine structure mapping is often required to accurately locate QTLs to chromosome regions, to estimate QTL effects, and for QTL cloning and gene function studies. Fine mapping in the Steptoe/Morex cross of a chromosome 1 (7H) multiple malting quality trait QTL region reduced the interval from 46 cM to 27 cM; the smallest individual QTL interval between molecular markers in the larger QTL region was decreased from 1.1 cM to 0.3 cM (Han et al. 1997b). Fine mapping the dormancy SD1 QTL suggested that there may be a gene cluster of at least three genes in the original QTL region, and the SD1 complex was resolved into a 4.4-cM region near the chromosome 7 (5H) centromere (Han et al. 1999). QTL fine mapping also may improve molecular breeding for quantitative traits (Han et al. 1997a). Many published QTLs span more than 20 cM along chromosomes and could include genes with undesirable effects. Gross QTL location may lead to low efficiency in quantitative trait breeding, as undesirable linkage drag may occur during molecular marker-assisted QTL transfer in breeding lines.

When SD2 was verified, only four restriction fragment length polymorphism (RFLP) markers were available in the 8-cM chromosome 7 (5H)L telomere target region (Han et al. 1996). The gene composition of SD2 was unknown, and both its location and effects were only approximately estimated. It is possible to locate SD2 more accurately to study its inheritance more precisely with an improved molecular map and improved QTL analysis techniques. SD2 is an interesting dormancy QTL for barley breeding because it promotes a moderate seed dormancy effect and is expressed under multiple growing conditions. The fine mapping of SD2 would provide useful genetic information and would have promising breeding applications.

High-resolution mapping of QTLs can be achieved through three approaches – mathematical, recombinational, and substitution mapping (Paterson 1998). In our laboratory, the substitution mapping method has been successfully employed as noted above (Han et al. 1997b, 1999). Here, we present fine mapping of the SD2 QTL using the substitution mapping approach.

Materials and methods

Plant materials

Steptoe is a six-row high-yielding feed barley cultivar with high seed dormancy (Muir et al. 1973; Han et al. 1996). Morex is a six-row, North American malting industry standard malting barley cultivar with low seed dormancy (Rasmusson et al. 1979; Han et al. 1996). A population of 150 Steptoe/Morex doubled haploid lines (DHLs), generated from F_1s by a modification of the *Hordeum bulbosum* method (Chen and Hayes 1989), was employed to construct an initial barley molecular genetic map (Kleinhofs et al. 1993). The DHLs in this population were employed in the original detection (Ullrich et al. 1993; Oberthur et al. 1995) and verification of SD2 (Han et al. 1996).

A set of isolines was developed for substitution mapping. To facilitate and accelerate the process of constructing Morex isolines, we used a molecular marker-assisted backcrossing strategy. From the original mapping population, DHL-59, which has a relatively high percentage of Morex genetic background (65%), was selected to backcross to Morex. DHL-59 carries the Steptoe segment containing SD2 and carries Morex segments in the other three – i.e., SD1, SD3 and SD4 – seed dormancy QTL regions. Molecular markers mapped within the SD2 region (http://barleygenomics.wsu.edu/Macdraw/7-150.jpg) were used to dissect the region and to facilitate selection for desirable genotypes in the backcrossing process. Because the original SD2 mapping was approximate, the ABC309-MWG851 region was extended to the ABG495A-MWG851B interval to insure inclusion of the QTL (Fig. 1). The entire ABG495A-MWG851B interval studied spans about 44 cM. In order to obtain Morex isolines with a clean Morex genetic background, we checked at least one RFLP marker per 20–30 cM in map regions where DHL-59 carried Steptoe segments after each backcross. All available markers in the ABG495A-MWG851B interval (http://barleygenomics.wsu.edu/Macdraw/7-150.jpg) were checked. Morex was regenerated (RGM) through the same backcrossing process. The BC_2F_1 and BC_3F_1 populations were grown under photoperiod and temperature control in a growth room to postpone flowering time in order to facilitate the selection of plants with desirable genotypes for selfing or further backcrossing. Before genotyping, the plants were grown under a day/night regime of 12 h, 25 °C/12 h, 15 °C, respectively; after genotyping, the growth conditions were adjusted to 16 h of light at 30 °C and 8 h of darkness at 25 °C to accelerate flowering time. Genotyping, selection, and selfing generally occurred through to the BC_3F_2 population. However, except for D8 and D9, all Morex isolines homozygous for certain Steptoe segments were confirmed in BC_3F_3 plants because the dominant molecular markers MWG851B, MWG851C, and MWG851D could not be determined in heterozygotes. For experimental control, two to five duplicate lines of each type of Morex isoline (except for isoline type D9) were planted, each with 16–25 seeds, and genotyped. Only homozygous lines (Table 1) were used in seed germination tests and statistical analyses.

Growing conditions

The experiments were conducted in two consecutive years. All Morex isolines (BC_3F_3), together with Steptoe and Morex, were randomly arranged and grown in the field at Pullman, Washington and in a growth room during the summer of 2000 and in the field in 2001. During the seed development periods in the field, maximum air temperatures reached 38 °C and 38.5 °C, with average daily highs of 28 °C and 30 °C in 2000 and 2001, respectively (http://www5.ncdc.noaa.gov/pdfs/cd/washington). The growth room was maintained at a photoperiod of 14/10 h (day/night) and day and

Table 1 Morex BC_2F_2 isolines and their duplicates

Isolines	D1	D2	D3	D4	D5	D6	D7	D8	D9	RGM
Duplicate accession nos.	109 113	210 213	307 308 309 314 319	415 418 422 429	502 506 510 513	615 616 623 633	705 707 725 731	802 804 805 808	824	601 711

night temperatures of 25 °C and 15 °C, respectively. To assure seed dormancy development, we employed lower growing temperatures in the growth room than those expected in the field.

Germination tests

Seeds were harvested at physiological maturity (as determined when the green color had disappeared from the spike). Heads were collected and stored in a –20 °C freezer prior to germination tests of the seeds. Germination tests were carried out after two different post-harvest after-ripening periods at room temperature had been applied: 0 days and 7 days for materials grown in 2000, and 1 day and 8 days for materials grown in 2001. Three replications of 50 seeds were germinated at 30 °C for each after-ripening period. Standard germination tests were performed (AOSA 1988). Germinated seeds were counted on the 7th day of germination, and seed germination percentages (GP) were calculated as the number of germinated seeds divided by the total number of seeds tested × 100.

QTL-effect analysis

The principle of substitution QTL fine mapping has been described in previous publications (Han et al. 1997b, 1999). In brief, all Morex isolines had a similar Morex genetic background and, theoretically, only differed genotypically within the ABG495A-MWG851B interval (Fig. 1). The differences among GPs should result from the differences of Steptoe segments carried in this interval. By comparing the GP values from different Steptoe segments, the dormancy QTL should be precisely located. If similar dormancy effects are found with several Morex isolines, a dormancy QTL is located in the Steptoe segment common among those lines; if similar dormancy effects are found with several Morex isolines, but no overlapping Steptoe segment exists among those lines, a dormancy QTL is located to any unique Steptoe chromosome segment(s) that those lines carry. GP values of Morex isolines were used to represent the dormancy effects of the Steptoe segments that the Morex isolines carried. Analyses of variance (ANOVA) of GP values were performed using SAS program version 6.12 (SAS 1996) on experiments carried out in 2000 in the growth room and in 2000 and 2001 in the field. If there was no significant difference among experiments, the combined dormancy effects of each Steptoe segment are presented. If significant genotype × experiment interaction was found, ANOVA was performed separately for each experiment, and results from each experiment were compared and combined. GP values of all Morex isolines (Table 1) were analyzed with Duncan's new multiple range test (NMRT) using SAS program version 6.12 (SAS 1996). To better control the genetic background, an average GP for duplicates of each type of Morex isoline (Fig. 1) was calculated, because it was not possible to determine the exact purity of the Morex genetic backgrounds. If there was no significant GP difference between Morex and RGM, the GP values of all Morex isolines were compared to that of RGM by the NMRT, otherwise to the GP value of Morex.

Results

Genotypes and duplicates of isolines

Ten different types of Morex isolines were constructed and designated D1–D9 and RGM. Their genotypes in the chromosome 7 (5H) ABG495A-MWG851B interval are depicted in Fig. 1. Isolines D1–D7 carry increased an length of the Steptoe segment in the direction from MWG851B to ABG495A; D8 carries a Steptoe segment from ABG390 to ABG495A; D9 only carries a Steptoe segment for marker ABG390, and all other markers in the MWG851B-ABG495A region have Morex alleles. The background genome for all isolines is Morex. RGMs are regenerated Morex lines that showed Morex genotype patterns for all of the molecular markers checked throughout the genome after the molecular marker-assisted backcrossing processes. For experimental control, a duplicate(s) of each Morex isoline was (were) generated, except for isoline type D9 (Fig. 1, Table 1).

Statistical analysis of experiments, replications and duplicate isolines

No significant GP differences were found among replications within each experiment. Significant genotype × experiment interactions were found across experiments. Accordingly, ANOVA was performed separately for each experiment. In some cases, complicated ANOVA results among duplicates within isoline type were observed (Table 2). A general GP value comparison among duplicates within each Morex isoline at $\alpha = 0.01$ level is:

(1) 2000 field, 0-day GP test: no significant differences among duplicates were found for isoline series D1, D4, D5, D8 and RGM, while some differences were found among duplicates for series D2, D3, D6 and D7.
(2) 2000 field, 7-day GP test: no differences were found among duplicates for any series except between the two D2 lines.
(3) 2001 field, 1-day GP test: no differences among duplicates were found for series D1, D2, D3, D4 and D8, and some differences were found among duplicates for series D5, D6, D7 and RGM.
(4) 2001 field, 8-day GP test: no differences were found among duplicates for any series except for isolines of D5 and D6.

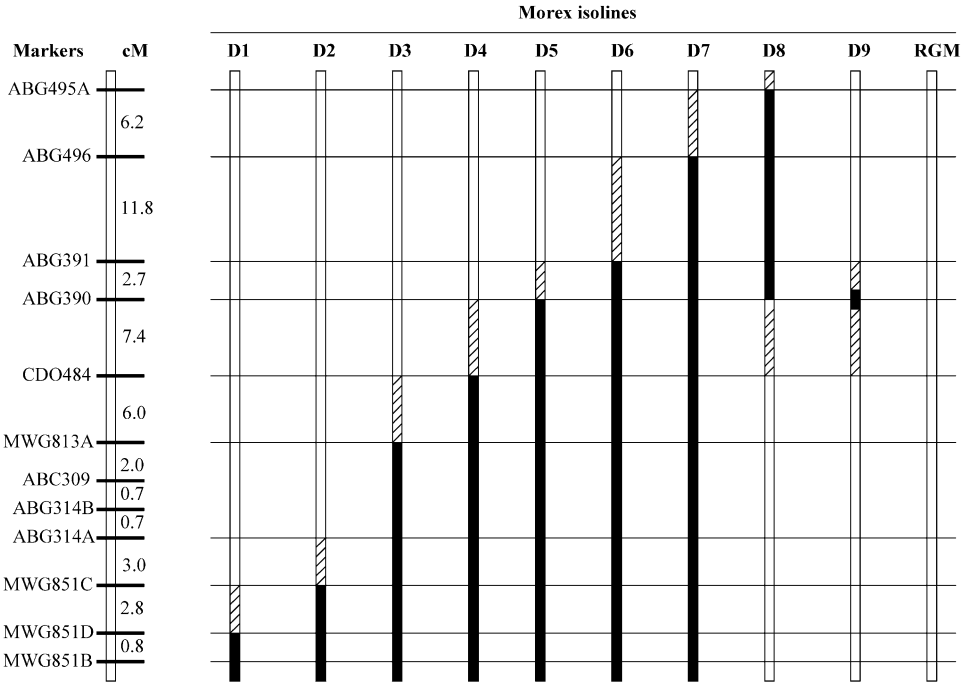

Fig. 1 Genotypes of the Morex isolines. Ten different Morex isolines designated *D1* through *D9* and regenerated Morex (*RGM*) were constructed for the chromosome 7 (5H) ABG495A to MWG851B region. *Open bars* represent Morex segments, *solid bars* represent Steptoe segments, *hatched bars* represent uncertain locations of crossovers

Table 2 Duncan's new multiple range test results for germination percentage at each afterripening period for duplicate Morex isolines. Morex isoline type D9 was not included because it does not have a duplicate

Isoline		2000 Field		2001 Field		Growth room	
		0 Day	7 Days	1 Day	8 Days	0 Day	7 Days
D1	109	A	A	A	A	A	A
	113	A	A	A	A	A	A
D2	210	B	B	A	A	A	A
	213	A	A	A	A	B	A
D3	307	B	A	A	A	B	A
	308	A	A	A	A	A B	A
	309	A	A	A	A	A	A
	314	A B	A	A	A	A	A
	319	A B	A	A	A	A B	A
D4	415	A	A	A	A	A	A B
	418	A	A	A	A	A	A B
	422	A	A	A	A	A	A
	429	A	A	A	A	A	B
D5	502	A	A	A B	A B	A	A
	506	A	A	B	B	A	A
	510	A	A	B	A B	A	A
	513	A	A	A	A	A	A
D6	615	A B	A	B	B	B	A
	616	B	A	A	A B	A B	A
	623	A	A	A	A	A	A
	633	A	A	B	B	A B	A
D7	705	A	A	B	A	A	A
	707	A	A	A B	A	A	A
	725	A	A	A	A	A	A
	731	B	A	A	A	A	A
D8	802	A	A	A	A	A	A
	804	A	A	A	A	A	A
	805	A	A	A	A	A	A
	808	A	A	A	A	A	A
RGM	601	A	A	A	A	A	A
RGM	711	A	A	B	A	A	A

Duplicates with the same letter are not significantly different at $\alpha = 0.01$ level

Table 3 Germination percentages at two after-ripening periods of Steptoe, Morex, regenerated Morex (RGM), and Morex isolines grown in three environments

	Growth room		2000 Field		2001 Field	
	0 Day	7 Days	0 Day	7 Days	1 Day	8 Days
Steptoe	34.7**	33.3**	34.0**	53.3**	21.9**	28.0**
Morex	83.3	98.0	79.3	92.7	93.3	100.0
RGM	87.7	99.7	83.0	95.2	81.2	96.7
D1	65.3**	88.7**	80.3	97.0	76.4	98.3
D2	68.0**	90.7*	56.0**	72.0**	74.9	97.3
D3	71.2**	91.1*	80.0	94.8	83.3	97.9
D4	71.5**	90.5*	81.2	95.8	77.3	94.3
D5	76.7*	89.5**	77.5	92.5	68.4	94.5
D6	72.0**	93.0	75.3	94.2	60.7**	86.3**
D7	67.8**	90.0**	64.2**	88.8	71.4	96.8
D8	80.7	99.3	74.8	95.8	77.9	93.3
D9	80.0	99.3	86.0	96.0	89.3	97.3

*, ** NMRT significant difference at $\alpha = 0.05$ and 0.01, respectively, for isolines and parents versus RGM

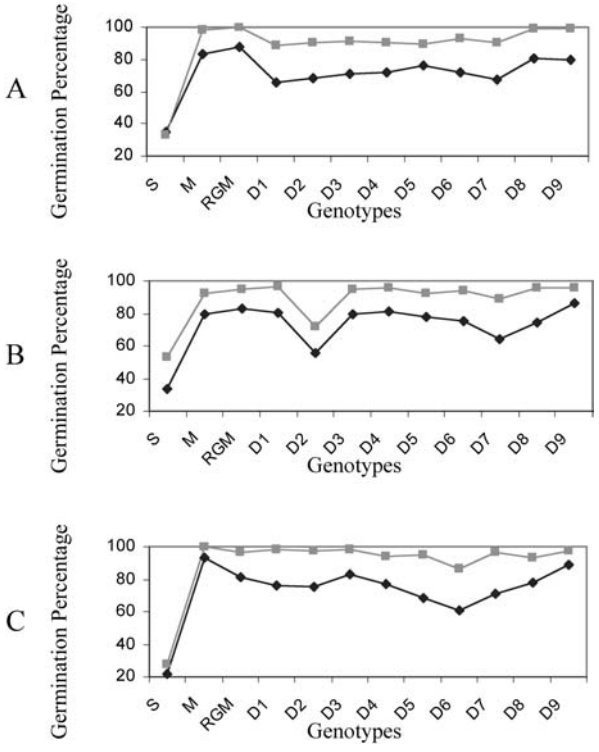

Fig. 2A–C Genotype versus germination percentage changes in different post-harvest periods and different growing environments. **A** Data from growth room, **B** data from 2000 field, **C** data from 2001 field. *Diamonds* 0-day or 1-day post-harvest period, squares 7-day or 8-day post-harvest period. *S* Steptoe, *M* Morex, *RGM* regenerated Morex, *D1–D9* Morex isolines

(5) Growth room, 0-day GP test: no differences were found among duplicates within isoline series except for series D2, D3 and D6.
(6) Growth room, 7-day GP test: differences were found only between two duplicates for D4.

In general, no differences in GP were found among duplicates for isoline series D1 and D8 and isoline D9 in any test situation, and none of the Morex isoline types showed differences among duplicates in six germination tests. Even though significant genotype × experiment interaction was found, no significant differences were found among the three growing environments across post-harvest after-ripening periods (Fig. 2), if the GP means of duplicates for each type of isoline were used to represent the GP of each isoline type. Accordingly, duplicate isoline means for each experiment were used in mean separation tests for QTL fine mapping (Table 3).

SD2 fine mapping

There was congruence between RGM and Morex for GP across all tests (Table 3). Therefore, the RGM mean was used as the control in fine mapping dormancy QTL SD2. In the growth room, 0-day GP test, D1, D2, D3, D4, D5, D6, and D7 were significantly different from RGM, so a QTL could be located in the 0.8-cM MWG851D-MWG851B interval. In the field 2000, 0-day GP test, D2 and D7 were highly significantly different from RGM. The difference between RGM and D2 and D7 should come from the Steptoe segments that D2 and D7 carried, and the dormancy QTL could be located to the overlapped Steptoe segment for D2 and D7 – that is, the 3.6-cM MWG851C-MWG851B interval (Fig. 1). In the field 2001, 1-day GP test, D6 was highly significantly different from RGM. The difference between RGM and D6 should come from the Steptoe segments that D6 carried, and the dormancy QTL could be located to the Steptoe segment in the 26.1-cM ABG391-MWG851B interval. Therefore, on the basis of all the data from the 0-day and 1-day GP tests under growth room and field conditions over the 2 years, a dormancy QTL is most likely to be located in the common 0.8-cM interval between markers MWG851D-MWG851B.

Although the GPs generally rose sharply from 0- or 1-day to 7- or 8-day after-ripening, the 7- and 8-day GP data generally confirmed the 0- and 1-day results that a dormancy QTL is most likely located in the MWG851D-MWG851B interval. In the 2000 field experiment after a 7-day after-ripening period, there also is an indication that

there may be an additional QTL located in the ABG496-MWG851C interval that might reduce the expression of the dormancy QTL in the MWG851D-MWG851B interval during after-ripening, since the GP difference between D7 and RGM present at 0-day post-harvest disappeared after 7-days post-harvest.

Based on the combined results of all six measures of GP (dormancy), a dormancy QTL is most likely located in the 0.8-cM interval between markers MWG851D and MWG851B, in the telomere region of chromosome 7L. There may possibly be another dormancy QTL located in the ABG496-MWG851C interval, which might accelerate the reduction of the dormancy effect of the QTL in the MWG851D-MWG851B interval during post-harvest after-ripening.

Discussion

Duplicate isolines and multiple environments

Testing in multiple locations and years with duplicate isolines should reduce experimental errors. This is especially important for any genetic study of quantitative traits that are controlled by a number of genes, environments, and the interaction between genes and environments. However, often it is difficult to carry out an experimental design employing multiple locations, years, replications, and duplicate isolines in QTL fine mapping research because of two major limitations: availability of seed and the amount of work necessary to generate duplicate isolines. Increasing seeds and using seeds from higher generations could partially overcome the seed source limitation. This was successfully employed in previous QTL fine mapping (Han et al. 1997b) in which consistent results were obtained from using seeds of two generations in 2 different years. Both duplicate isoline and multiple environment strategies were used in the present study as well. No significant difference was found among the three experimental environments, but significant differences were sometimes found among duplicates of certain isolines. Significant genotype × environment interactions were also found across the 2 years of experiments.

Based on the 2000 and 2001 field data separately, a dormancy QTL could only be located in the 3.6-cM interval between markers MWG851C and MWG851B and the 26.1-cM interval between markers ABG391-MWG851B, respectively. By combining field data from 2 years we could locate the dormancy QTL into the 3.6-cM MWG851C-MWG851D interval. But by combining all field and growth room data for the 2 years, we were able to narrow down this QTL into the MWG851D-MWG851B interval, which only spanned 0.8 cM (Table 3, Fig. 1). In addition, consistencies between the results from both the field and growth room experiments increased our confidence in drawing conclusions from this study (Fig. 2). The location of the SD2 QTL between the last two markers at the end of the chromosome (MWG851D-MWG851B) is further supported by the original QTL analyses (interval mapping) in that the test statistics from two different interval mapping methods were greatest in the last marker interval of the chromosome (or in other terms, the QTL curve was still rising at the end of the chromosome) (Ullrich et al. 1993; Oberthur et al. 1995).

The accuracy of this research was also increased by enhanced experimental error control through using duplicate isolines. However, duplicate isolines and multiple environments can also complicate genetic studies. In this investigation, significant differences were found among duplicates of certain isolines but, in general, these differences were often not consistent among experiments. These complicated results among duplicates could be explained by: (1) even though the genetic background of duplicate isolines appeared to be the same based on molecular marker-assisted genotyping data, there might be genetic differences because it is impractical to check the whole genetic background completely; (2) even within the dissected target region, genotypic differences between duplicate isolines could be possible based on an imperfect detection of crossover points. For example, in the present study, two D2 lines, 210 and 213, showed the same genotype for all of the molecular markers checked, but their GPs were significantly different from each other ($\alpha = 0.01$) in samples from growth room, 0-day, field, 0-day, and field, 7-days, while no significant difference was found from field, 1-day, field, 8-day, and growth room, 7-day samples. Two possible explanations are that: (1) they might still be different in the genetic background outside the ABG495A-MWG851B region; (2) they might be different within the ABG314A-MWG851C interval where genotyping was limited because of molecular marker availability in the crossover interval (Fig. 1). In spite of the rigors of the experimental procedures, experimental error could have contributed to the unexpected differences as well. Thus, the average GP value was assessed to be more representative than a single line value of an isoline type.

Temperature vs dormancy QTL complexity

A relatively low temperature during seed development can enhance the expression of dormancy genes, but the mechanism is not clear (King 1989). In this study, Morex isolines were grown under both field and growth room conditions. Relatively lower growing temperatures were set in the growth room than those experienced in the field to enhance dormancy development. A higher and more consistent expression of the dormancy QTL in the MWG851D-MWG851B interval was found in growth room-grown seed than in the field-grown seed. The average and highest temperatures in the field during seed development were lower in 2000 than in 2001, and the 2000 data located the dormancy QTL into a narrower chromosomal region than the 2001 data. These results suggest that low temperature had an effect on the

dormancy QTL in the MWG851D-MWG851B interval. Both isolines D1 and D2 carried the critical MWG851D-MWG851B interval, but a low temperature was required to express significant seed dormancy in D1 (Table 3). No significant seed dormancy was detected in field-grown seed of D1. However, in D2, significant dormancy was detected from seed grown in both the lower temperature regime of the growth room and the higher temperature field regime of the field in 2000. One possible explanation is that although a high temperature suppressed the expression of the seed dormancy QTL in the MWG851D-MWG851B interval, this suppression could possibly be removed by the action of a putative gene(s) from Steptoe located in the MWG851C-MWG851D interval. However, no significant dormancy was detected in D2 from higher temperature, field-grown seed in 2001. The putative gene(s) may not respond or a different set of genes may be involved in the expression of the QTL in the MWG851D-MWG851B interval when the environmental temperatures change to a certain level. The effect of the region above MWG851D-MWG851B was also supported by other results in the present study. Lines D1–D7 all carry the Steptoe MWG851D-MWG851B segment, but only D6 consistently expressed high seed dormancy in 2001 field seed. We suggest that the expression of dormancy QTL in the MWG851D-MWG851B interval was in general, temperature-dependent and that the chromosomal region immediately above it, the ABG496-MWG851D interval, may have a putative gene(s) that has (have) effects on its expression.

Prospects for genetic manipulation of seed dormancy

In barley cultivar development, moderate seed dormancy is desirable (Han et al. 1999; Romagosa et al. 1999). High seed dormancy could lead to non-uniformity in seed germination and could create a weed problem in succeeding crops, while low seed dormancy could lead to sprouting in the head and reduced seed value (Ullrich et al. 1993). In the Steptoe/Morex cross, four major seed dormancy QTL regions have been identified to date and complicated interactions among these QTLs detected (Han et al. 1996; Romagosa et al. 1999). A larger number of minor QTLs have also been detected in limited environmental situations (Ullrich et al. 1993). Cytoplasm might also have effects on barley seed dormancy (Han et al. 1996).

It has been difficult for breeders to manipulate seed dormancy genes. Dormancy QTL mapping may facilitate manipulation of dormancy genes with the assistance of molecular markers. SD2 has a moderate seed dormancy effect, which makes it a promising candidate dormancy gene for barley breeders. In the investigation reported here, in which molecular marker-assisted backcrossing was applied, different chromosome segments containing a seed dormancy QTL were successfully transferred from Steptoe to Morex. The transferred dormancy QTL was generally expressed best under growth room growing conditions, while under field growing conditions, the dormancy effect was detected in only some of the Morex isolines. Therefore, the expression of this QTL appears to be environmentally sensitive, a characteristic expected of most dormancy QTLs. Our research supports the complexity of gene composition and expression of dormancy QTL in the chromosome 7 long-arm telomere region and suggests that an additional gene(s) might be required for the appropriate expression level of the dormancy QTL in the MWG851D-MWG851B interval. Further studies are required to understand the gene composition and regulation of the seed dormancy QTL in the MWG851D-MWG851B interval so as to efficiently manipulate this QTL in practical barley breeding programs.

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References

AOSA (1988) Association of Official Seed Analysis rules for testing seeds. J Seed Technol 12, issue 3

Chen F, Hayes PM (1989) A comparison of *Hordeum bulbosum*-mediated haploid production efficiency in barley using in vitro floret and tiller culture. Theor Appl Genet 77:701–704

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, Romagosa I, Ullrich SE, Jones BL, Hayes PM, Wesenberg DM (1997a) Molecular marker-assisted selection for malting quality traits in barley. Mol Breed 3:427–437

Han F, Ullrich SE, Kleinhofs A, Jones B, Hayes PM, Wesenberg DM (1997b) Fine structure mapping of the barley chromosome 1 centromere region containing malting quality QTLs. Theor Appl Genet 95:903–910

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

King RW (1989) Physiology of sprouting resistance. In: Derera NF (ed) Preharvest field sprouting of cereals. CRC Press Boca Raton, pp 27–55

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, 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

Muir CE, Nilan RA (1973) Registration of Steptoe barley. Crop Sci 13:770

Oberthur LE, Dyer W, Ullrich SE, Blake TK (1995) Genetic analysis of seed dormancy in barley (*Hordeum vulgare* L.). J Quant Trait Loci/J Agric Genomics (online) http://www.ncgr.org/jag/papers95/paper595/indexp595.html

Paterson AH (1998) High-resolution mapping of QTLs. In: Paterson AH (ed) Molecular dissection of complex traits. CRC Press, New York, pp 163–173

Rasmusson DC, Wilcoxson RD (1979) Registration of 'Morex' barley. Crop Sci 19:293

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

SAS Institute (1996) SAS user's guide, version 6.12. SAS Institute, Cary, N.C.

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, Ried JL (eds) Preharvest spouting in cereals 1992. Am Assoc Cereal Chem, St Paul, Minn., pp 136–145

Ullrich SE, Han F, Blake TK, Oberthur LE, Dyer WE, Clancy JA (1996) Seed dormancy in barley. Genetic resolution and relationship to other traits. In: Noda K, Mares D (ed) Preharvest sprouting in cereals 1995. Center for Academic Societies, Osaka, Japan, pp 157–163

Ullrich SE, Han F, Jones BL (1997) Genetic complexity of the malt extract trait in barley suggested by QTL analysis. J Am Soc Brew Chem 55:1–4