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Verification of barley seed dormancy loci via linked molecular markers

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Abstract Seed dormancy is a relatively complex trait in barley (*Hordeum vulgare* L.). Several dormancy loci were identified previously by quantitative trait locus analysis. Three reciprocal crosses were made in the present study between parents carrying specific dormancy alleles via linked molecular markers to verify individual dormancy locus effects and potential expression. Analyses of F_2 progenies revealed that the dormancy allele at the locus flanked by the markers *Ale* and ABC302 on the long arm of chromosome 7 had a major effect on dormancy, and was at least partly epistatic to the dormancy locus in the ABC309–MWG851 interval near the telomere of the long arm of chromosome 7. In the absence of the dormancy allele in the *Ale*–ABC302 interval, the allele in the ABC309–MWG851 interval exerted moderate to large effects on dormancy. Cytoplasmic effects on dormancy were also observed. The germination percentages of progeny with relatively high levels of dormancy were more variable than those of non-dormant or less-dormant progeny, apparently due to environmental effects. Removal of the dormancy allele in the *Ale*–ABC302 interval, or introducing the dormancy allele in the ABC309–MWG851 interval, should suffice for adjusting dormancy levels in breeding programs to suit various production situations and end uses. The verification of dormancy loci via linked molecular markers allows manipulation of these loci in applied breeding programs.

Key words *Hordeum vulgare* · Dormancy · Quantitative trait loci · Molecular marker-assisted selection

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Introduction

Seed dormancy is described as the inability of a viable embryo to germinate under environmental conditions favorable to germination. Levels of dormancy may be variable and are determined by the interactions among plant genotype; environmental conditions at the time of seed development, harvest, storage and germination; microbial activities associated on and within seeds; and the germination test employed (Hareland and Madson 1989).

Post-harvest dormancy in barley (*Hordeum vulgare* L.) seed can be either beneficial or detrimental to production and end use. Dormant seed may require storage time prior to re-planting or malting, and can also create a weed problem in succeeding crops (Ullrich et al. 1993). Non-dormant seed can often result in pre-harvest sprouting in the field which can reduce malting, seed, and feed quality. Therefore, breeding for an adequate level of dormancy in barley could be very significant. Since the levels of dormancy in barley vary widely in response to environment and genotypic constitution (Buraas and Skinnes 1984; Strand 1989), phenotypic selection for or against dormancy could be rather difficult. An alternative approach has been proposed by selecting quantitative trait loci via linked molecular markers (Lande and Thompson 1990). If linkage between a locus of interest and flanking markers is detected, the markers can be used as tags to follow and select the desirable allele in segregating populations. However, the effectiveness of molecular marker-assisted selection (MMAS) in plant breeding has not been adequately demonstrated.

Dormancy is generally grouped into two categories: embryo dormancy and coat-enhanced dormancy, and therefore is under rather complex genetic control (Bewley and Black 1994). A previous genetic study revealed that dormancy in barley may be governed by several recessive genes which are expressed at different pre-harvest and after-ripening stages, with no apparent

cytoplasmic effects (Burass and Sinnes 1984). Recently, with the development of molecular markers and mapping techniques for quantitative trait loci (QTLs), systematic mapping and manipulation of dormancy loci have become possible. Based on a 'Steptoe'/'Morex' doubled-haploid line (DHL) population map, constructed by the North American Barley Genome Mapping Project (Kleinhofs et al. 1993), a number of dormancy loci were detected by QTL analyses with four loci found in common (Ullrich et al. 1993; Oberthur et al. 1995). These four loci (designated SD1–4 in this study) flanked by the following RFLP markers are: SD1 between *Ale* and ABC302 on the long arm of chromosome 7, SD2 between ABG309 and MWG851 at the end of the long arm of chromosome 7, SD3 between *Amy2* and *Ubi1* on chromosome 1, and SD4 between WG622 and BCD402B on chromosome 4. SD3 and SD4 were identified only in specific environments. These four loci accounted for approximately 50, 15, 5 and 5% of the variability in seed dormancy, respectively (Ullrich et al. 1993; Oberthur et al. 1995).

After identifying putative QTLs of interest, the next important step in applying QTL mapping information is to validate the identified QTLs in terms of actual heritable genes, and to determine the practical value for selection and manipulation in breeding programs. The objectives of the present study were: (1) to verify the major putative dormancy loci (SD1 and SD2) on chromosome 7 via realized selection response; and (2) to explore possible interactions between SD1 and SD2.

Material and methods

Establishment of segregating populations

Six Steptoe/Morex DHLs were chosen as parents based on the allelic state of the SD1 locus. Steptoe, a 6-row spring feed cultivar, has been a dominant barley cultivar in the Pacific Northwestern United States, but it expresses high levels of dormancy. Morex is the U.S.A. 6-row malting cultivar standard and it expresses no dormancy. The cytoplasm and genotypes at the SD1 and SD2 loci of these six DHLs are presented in Table 1. The *Ale*–ABC302 interval bracketing SD1 is 10.1 cM, and the ABC309–MWG851 interval bracketing SD2 is 8.0 cM. Since double crossovers are rare within these distances, we assume that the genotypes of the flanking markers represent the genotypes of the SD1 and SD2 loci. DH165 has the Morex allele at *Ale* and the Steptoe allele at ABC302 flanking SD1. The genotype of SD1 in DH165 was estimated by progeny analysis. Three reciprocal crosses, DH87/DH147 (designated RCA1) and DH147/DH87 (RCA2), DH129/DH64 (RCB1) and DH64/DH129 (RCB2), and DH91/DH165 (RCC1) and DH165/DH91 (RCC2), were made in the greenhouse in the summer of 1993. The F_1 seeds from all three reciprocal crosses were planted in the greenhouse in the winter of 1993/1994. The F_2 seeds from each F_1 head from the three reciprocal crosses were planted in 3-m rows in the field at Pullman, Washington in the summer of 1994. The six parents, DH87, DH147, DH129, DH64, DH91, and DH165, and Steptoe and Morex were also planted in individual rows as checks. The number of rows (equivalent to number of replications for each population) from each cross varied from 50 to 91. A total of 438 progeny rows were grown.

Germination tests

Intact F_2 heads (10–15) from each row were harvested by hand at physiological maturity and stored at −10 °C to arrest all physiological activity until germination analysis. Germination tests were performed on the F_3 seed from the harvested heads of each F_2 row. Germination tests were made at 30 °C with no after-ripening period, using two replications of 100 seeds. Standard germination test procedures (AOSA 1988) were followed. After 7 days, the number of germinated seeds were counted and expressed as a percentage of the total.

Statistical analysis

The mean germination percentages (GP) of the two replicates from all rows of the six populations (RCA1, RCA2, RCB1, RCB2, RCC1 and RCC2) were analyzed using SAS/STAT procedures (SAS 1991). The total sum of squares for GP was partitioned among the mean of the three sets of reciprocal crosses, differing in allele composition at the SD1 and SD2 loci (RCA, RCB, and RCC), and within each cross (RCA1 vs RCA2, RCB1 vs RCB2, and RCC1 vs RCC2) to determine if cytoplasmic effects were involved using combined analyses of variance.

Verification of progeny genotypes

In order to verify the allelic state at the SD1 and SD2 loci in the progeny of the three reciprocal crosses, bulk segregant analysis was performed (Michelmore et al. 1991). Rows with the 10% highest and 10% lowest GP (HGP and LGP, respectively) from each cross were identified and an F_3 seed from each selected row was planted in the greenhouse. Leaf tissue was harvested from each of these plants, frozen immediately in liquid nitrogen, lyophilized for 3 days, and stored at −20 °C until analysis. An equivalent amount of leaf tissue from the plants representing the 10% highest or 10% lowest GP rows was bulked for DNA extraction. The DNA preparation, digestion, transferring and filter hybridization have been described by Kleinhofs et al. (1993). The bulks were screened with the following markers: *Ale*, ABC302, ABG309 and MWG851.

Table 1 Selected Steptoe/Morex DHLs and Steptoe and Morex with their cytoplasm, allelic state of flanking markers for the SD1 and SD2 loci, and germination percentage (GP) (based on the germination test of seeds from 1994 field planting). S-Steptoe allele; M-Morex allele

DHL	Cytoplasm	SD1		SD2		GP
		Ale	ABC302	ABC309	MWG851	
DH87	Steptoe	S	S	M	M	19.0
DH147	Steptoe	S	S	M	M	22.5
DH64	Morex	M	M	M	M	55.5
DH129	Steptoe	M	M	S	S	44.4
DH91	Morex	M	M	M	M	35.5
DH165	Steptoe	M	S	M	M	28.3
Steptoe	Steptoe	S	S	S	S	21.5
Morex	Morex	M	M	M	M	94.5

Results

Genotypic and environmental effects

The mean , range, and coefficient of variation (CV) of GP for each cross are presented in Table 2. With both DH87 and DH147 having the Steptoe dormancy allele at the SD1 locus, the F_3 seeds of RCA1 and RCA2 showed a relatively low mean GP (22.4 and 22.1%, respectively). The parental GPs for DH87 (19.0), DH147 (22.5) and Steptoe (21.5) were similar to each other and to the RCA1 and RCA2 progeny, confirming that the SD1 locus has a major effect on dormancy. A relatively large range and high mean GP were observed for F_3 seed from RCB1 (60.4) and RCB2 (48.8) with the removal of the major Steptoe dormancy allele at the SD1 locus. But, the highest GP (87%) of the populations was less than the 94.5% of Morex. The GP of DH64 was 55.5, which was similar to the mean of RCB1, and the GP of DH129 was 44.4, which was similar to RCB2. The F_3 seeds derived from RCC1 and RCC2, which carry the Morex allele at *Ale* and both Steptoe and Morex alleles at ABC302, had relatively low GPs, with means of 40.0 and 28.5%, respectively. The mean GPs of the RCC populations were in between the mean GPs of the RCA and RCB populations. The GP of the parents, DH91 and DH165, were 35.5 and 28.3%, respectively, and similar to the means of RCC1 and RCC2.

Analysis of variance was performed to test the GP differences among and within the three reciprocal crosses (Table 3). The GP differences among the three reciprocal crosses were highly significant ($P \leqslant 0.01$). Significant differences also were found in GPs within the RCB and RCC reciprocal crosses. But no significant GP difference within the RCA reciprocals was detected.

Genotypes of bulks

In order to verify the expected allelic states of the SD1 and SD2 loci in the F_2 progeny derived from the three reciprocal crosses, bulk segregant analyses were performed. As expected, the bulks from RCA1 and RCA2 carried Steptoe alleles at the *Ale* and ABC302 loci, and Morex alleles at the ABC309 and MWG851 loci. The bulks from RCB1 and RCB2 carried Morex alleles at the *Ale* and ABC302 loci, and both Steptoe and Morex alleles at the ABC309 and MWG851 loci. The bulks from RCC1 and RCC2 had only the Morex alleles at the *Ale*, ABC309 and MWG851 loci. The HGP bulk of RCC1 had only the Morex allele at the ABC302 locus, and the HGP bulk of RCC2 had both Morex and Steptoe alleles at the ABC302 locus, but more individuals had the Morex allele based on RFLP band intensity. The LGP bulks of RCC1 and RCC2 had both Steptoe and Morex alleles at the ABC302 locus (Table 4).

Table 3 Analysis of variance of GP for the reciprocal crosses

Source	Degree of freedom	Mean square	F-value
Reciprocal crosses	2	43885	444.0**
Within reciprocal crosses	3	3007	30.4**
RCA1 vs RCA2	1	457	0.05
RCB1 vs RCB2	1	5403	54.7**
RCC1 vs RCC2	1	3614	36.6**
Error	432	98.8	

** Significance at the level of 0.01

Discussion

Dormancy locus effects

By manipulating selected dormancy loci via linked molecular markers, we were able to verify the mapping

Table 4 Bulk segregant analyses of three reciprocal crosses for selected marker loci. Each cross contains high germination percentage (HGP) bulk and low germination percentage (LGP) bulk. "S" means Steptoe allele, "M" means Morex allele, "SM" means that both Steptoe and Morex alleles exist in a bulk

Cross	Bulk	*Ale*	ABC302	ABC309	MWG851
RCA1	HGP	S	S	M	M
	LGP	S	S	M	M
RCA2	HGP	S	S	M	M
	LGP	S	S	M	M
RCB1	HGP	M	M	SM	SM
	LGP	M	M	SM	SM
RCB2	HGP	M	M	SM	SM
	LGP	M	M	SM	SM
RCC1	HGP	M	M	M	M
	LGP	M	SM	M	M
RCC2	HGP	M	sM[a]	M	M
	LGP	M	SM	M	M

[a] sM means that the intensity of Steptoe band is less than that of Morex band

Table 2 Mean, range, and coefficient of variation (CV) of germination percentage of F_2 progeny from three reciprocal crosses

Cross	Designation	Replications (rows)	Mean ± SE[a]	Range	CV(%)
DH87/DH147	RCA1	80	22.4 ± 0.7	11.0–37.0	28.6
DH147/DH87	RCA2	91	22.1 ± 0.9	6.5–35.5	37.1
DH129/DH64	RCB1	78	60.4 ± 1.6	34.0–87.0	22.6
DH64/DH129	RCB2	78	48.8 ± 1.5	23.5–86.5	27.0
DH91/DH165	RCC1	50	40.0 ± 1.2	24.5–63.5	21.8
DH165/DH91	RCC2	61	28.5 ± 0.7	12.5–40.0	20.4

[a] Standard error

results of the dormancy QTLs and demonstrate the expression of individual loci. The SD1 locus on chromosome 7 was confirmed to have a large impact on dormancy. The average GPs of F_3 seed of RCA1 and RCA2 homozygous for the Steptoe allele at SD1 were very close to the GP of Steptoe (about 22%) (Tables 1 and 2). With the removal of this large-effect Steptoe dormancy allele, the average dormancy level of F_3 seed of RCB1 and RCB2 was greatly reduced (GP increased), but did not reach the low level of Morex. These results suggest that the dormancy allele at the SD1 locus has a strong effect on the physiological pathway leading to the imposition, maintenance and/or release of dormancy and is at least partly epistatic to other dormancy loci (Oberthur et al. 1995). When this major dormancy allele is removed, the SD2 dormancy allele appears to exert a larger effect on dormancy (Table 2), since some rather dormant phenotypes are present in the RCB populations. The mean GPs of the RCC populations were between those of the RCA and RCB populations, indicating that the dormancy allele at the SD1 locus was segregating in the F_2 progeny. The Steptoe allele at the SD1 is probably present in DHL165. This suggests that the SD1 locus is more closely linked to ABC302 than *Ale*. The markers flanking the SD3 and SD4 loci were also screened in the bulks. SD 4 was segregating in the RCA populations, SD3 and SD4 were segregating in the RCB populations, and only the Steptoe allele was present at the SD3 locus in the RCC populations. The SD3 and SD4 loci might contribute some effects on the GPs of the RCA, RCB, and RCC populations. However, the overall GPs of these populations should not be greatly affected by the SD3 and SD4 loci, since SD3 and SD4 have been shown to have only minor effects on dormancy and were detected only in specific environments (Oberthur et al. 1995). They have not been detected in the Pullman environment previously (Oberthur et al. 1995). Based on the realized selection response, the selection of specific dormancy loci via linked molecular markers allowed for verification of individual locus effects and the detection of interactions (epistasis) among the loci.

The GP range of each reciprocal cross appeared to be related to the degree of the GP difference between the parents. However, the large range in GPs found in the RCB and RCC populations, and especially the presence of rather dormant phenotypes, indicates that, in addition to genetic factors, environmental effects might be involved as well. However, the sensitivity of different genotypes to the environment is variable. The greater GP CVs (average 32.9%) in the RCA populations (the most dormant, Table 2) might be an indication that there are more physiological or biochemical steps involved in bringing seeds into dormancy than into germinability. Thus, the expression of dormancy potential may be more sensitive to environmental factors. Recent evidence showed that the expression of certain genes was maintained in embryos of dormant, but not non-dormant, wheat grains (Morris et al. 1991). A cDNA clone has also been obtained using mRNA from hydrated, dormant embryos of *Bromus secalinus* which is absent from their non-dormant counterparts (Goldmark et al. 1992). Therefore, it would seem logical to suppose that the entry into dormancy, and subsequently the maintenance and release of dormancy, require additional steps or regulations compared to the entry into germinability.

Cytoplasmic effects

Significant GP differences between RCB1 and RCB2, and RCC1 and RCC2 (Tables 2 and 3) strongly suggest that cytoplasmic effects are involved in seed dormancy, which was not reported in the previous studies by Moorman (1942) and Buraas and Skinnes (1984). As a control, both RCA1 and RCA2 have the same cytoplasm, and indeed no cytoplasmic effects were detected. However, it appears that not all cytoplasmic effects on dormancy could be attributed to a specific cytoplasm, e.g., GP was lower with Morex cytoplasm in the RCB crosses, but higher with Morex cytoplasm in the RCC crosses (Table 2). However, since Morex is essentially non-dormant, we speculate that both Steptoe and Morex carry a cytoplasmic factor affecting dormancy, which must interact with a dormancy allele(s) in the nucleus to impose its effect. Such a situation is well documented with cytoplasmic male sterility resulting from the interaction of nuclear and cytoplasmic genes (Ahokas 1980). Therefore, different genome compositions, especially allelic states of dormancy loci, might result in different interactions between cytoplasmic factors and nuclear genes. Morex does not contain any dormancy alleles in the nucleus, thus cytoplasmic factors are inactive. Inconsistent results between our and previous studies presumably resulted from different germplasm (cytoplasms) used in the respective studies. Identification of a specific cytoplasmic factor may provide additional insights into the complexity of seed dormancy and germination processes. Maternal seed-coat effects on dormancy have been documented (Moorman 1942); but, since our dormancy analyses were based on F_3 seeds, no maternal effects would be detected.

Effectiveness of MMAS

Identification of linkage between dormancy loci and molecular markers by QTL analyses provides the opportunity for an alternative selection approach for or against dormancy via linked molecular markers. The SD1 and SD2 locus effects on dormancy, as validated in this study, would help to determine the target locus or loci for selection for specific breeding objectives. Selection for a high level of dormancy could be very effective by introducing the dormancy allele at the SD1 locus. However, selection for non-dormancy could not be

achieved by just removing the dormancy allele at the SD1 locus. As indicated above, for many applications a moderate level of dormancy is desirable. Therefore, a means to select for a moderate level of dormancy would be very useful. By removing the dormancy allele at the SD1 locus, or introducing the dormancy allele at SD2 locus, a moderate level of dormancy could be achieved.

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References

Ahokas H (1980) Cytoplasmic male sterility in barley. VII. Nuclear genes for restoration. Theor Appl Genet 57:193–202

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

Bewley JD, Black M (1994) Seeds: physiology of development and germination. Plenum Press, New York, pp 199–267

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

Goldmark PJ, Curry J, Morris CG, Walker-Simmons MK (1992) Cloning and expression of an embryo-specific mRNA up-regulated in hydrated dormant seeds. Plant Mol Biol 19:433–441

Hareland GA, Madson MA (1989) Barley dormancy and fatty acid composition of lipids isolated from freshly harvested and stored kernels. J Inst Brew 95:437–442

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes PM, Chen FQ, Lapitan N, Fenwich 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, isoenzyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Lande R, Thompson R (1990) Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Moorman B (1942) Untersuchungen uber Keimruhe bei Hafer und Gerste. Kuhn-Arch 56:41–79

Morris CF, Armstrong RJ, Goldmark PJ, Walker-Simmons MK (1991) Molecular cloning and expression of abscisic acid-responsive genes in embryos of dormant wheat seeds. Plant Physiol 95:814–821

Oberthur LE, Dyer W, Ullrich SE, Blake TK (1995) Genetic analysis of seed dormancy in barley (*Hordeum vulgare* L.). J Quant Trait Loci [Online]. Available: World Wide Web: http://probe.nalusda.gov:8000/otherdocs/jgtl1995–05/dormancy.html.1995

SAS Institute (1991) SAS user's guide. Statistics. Version 6.10. SAS inst, Cary, N.C., USA

Strand E (1989) Studies on seed dormancy in small grain species. I. Barley. Norwegian J Agric Sci 3:85–99

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) Pre-harvest sprouting in cereals 1992. Am Assoc Cereal Chem, St. Paul, Minn, USA, pp 136–145