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Quantitative trait loci for seed dormancy in wild barley (*Hordeum spontaneum* C. Koch)

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Abstract

A quantitative trait locus analysis was carried out to unravel the genetic basis of dormancy in wild barley (*Hordeum spontaneum*) from Israel. Two accessions, Ashkelon and Mehola, from divergent environments were crossed to produce a mapping population. A linkage map was produced from the F_2 population, and F_4 seeds were used for germination experiments. Five quantitative trait loci (QTL) were detected for dormancy across the different germination experiments. These QTL were found on chromosomes 1, 2, 5, 6 and 7. The variation explained by each QTL varied between 8 and 25%. Ashkelon alleles increased the germination except for the QTL on chromosome 5. Three out of these five QTL co-locate with QTL found earlier in cultivated barley crosses, although this does not necessarily imply that they would be the same loci. The level of dormancy is much higher in wild barley than in cultivated barley and wild barley may have alleles that have not yet been utilised in breeding for optimally dormant barley.

Introduction

Seed dormancy is an important trait not only for the agricultural industry but also for seedling survival in wild species. It has been well studied in several crop species, such as barley, wheat, oat and rice (for a review see Foley and Fennimore 1998). A seed is dormant when it fails to germinate even though it is mature and conditions for germination are optimal. Dormancy can be due to the embryo or the seed coat (Bewley and Black 1994). Embryo dormancy is lost first and therefore it is the seed coat dormancy that ultimately determines when the seed germinates (Rodriguez et al. 2001). Although non-dormant seeds are desirable in the malting industry, these might sprout in the ear in wet conditions before harvesting and therefore reduce yield and seed quality. Highly dormant seeds, on the other hand, create storage problems during their after-ripening period as well as being a weed problem.

The level of dormancy does not depend only on the genetic make-up of the seed but also on the environmental growing conditions of the maternal plant during the seed maturation process, although this seems to depend also on the cultivar (Schuurink et al. 1992). Germination success is also dependent on the environmental conditions during germination process. Especially temperature (Fennimore et al. 1998) and water saturation level (Thomas et al. 1996) play a crucial role.

Several quantitative trait loci (QTL) have been detected in cultivated barley for seed dormancy

(Ullrich et al. 1992, Oberthur et al. 1995, Han et al. 1996, Thomas et al. 1996). A major QTL (SD1) has been found on chromosome 7, explaining 36% of the variation in dormancy (Ullrich et al. 1992). In the same cross, three minor QTL were found on chromosomes 1, 4 and 7 (Ullrich et al. 1992).

Dormancy is much stronger in wild than in cultivated barley (Ogawara and Hayashi 1964; Gutterman et al. 1996). The removal of the glumellae and the husk enhance the germination greatly in wild barley (Gutterman et al. 1996; Wang 1997). This seems to be largely due to the increased abscisic acid (ABA) diffusion from the seed (Wang 1997). The duration of after-ripening in wild barley depends on the population; the more extreme the habitat the longer it takes for the dormancy to break (Gutterman and Nevo 1994). This has an important effect on seedling survival in drier areas where germination after unusual rain during the dry season would be fatal.

The aim of this study was to detect QTL for seed dormancy in wild barley and to compare the results to the QTL found in cultivated barley.

Material and methods

Plant material

A single pair cross between two accessions of *Hordeum spontaneum* C. Koch from Israel was made. The paternal plant was from the site Ashkelon (34°60′ E, 31°63′ N), on the Mediterranean coast, and mother plant from Mehola (35°48′ E, 32°13′ N), in the Jordan valley. We refer to the accessions as Ashkelon and Mehola, after the site of origin. Ashkelon has a moderate level of dormancy whereas Mehola is highly dormant, requiring a long after-ripening period. The Mediterranean coast, where Ashkelon is situated, is characterised by high humidity, low evaporation and a high number of dewy nights whereas Mehola has low humidity and high evaporation (Vanhala et al. 2004). F_1 and subsequently F_2 were left to self-fertilise. Ten F_3 seeds per one F_2 plant were pooled and grown together in a large (25 cm diameter) pot in order to produce F_4 seeds. In the remainder of this paper the bulked F_4 seeds deriving from a single F_2 plant are referred to as a line. The plants were grown in a non-heated greenhouse, sown autumn 1999 at the Ecological Institute, Heteren, The Netherlands.

The F_4 seeds were collected randomly from all the ten F_3 plants. Because wild barley has a brittle rachis, complete synchronisation of the seed ripeness was not possible. Instead, seeds were collected when they were getting loose from the ear. Already dropped seeds were excluded and seeds that were not loose were left to ripen further. The loose seeds were collected approximately every 3 days during a four-week period in June 2000 from 102 lines. The seeds were counted per line, left to air dry further at +15 °C, 30% humidity for approximately 3 days, then sealed in plastic bags and stored at −20 °C until the germination experiments could be started.

Germination experiments

Six germination experiments were conducted with 14-day intervals. Forty F_4 seeds per line were used for every germination experiment. The germination experiments were conducted using intact dispersal units, i.e. the husks were not removed. For the first germination experiment, seeds were left to thaw at room temperature over night before placing them on Petri dishes between two water-saturated blotting papers. The remainder of the seeds were put in paper envelopes and after-ripened in a dry oven at +40 °C. Batches of seeds were taken from these envelopes every 14 days to germinate them. The germination was done at +10 °C for five days then at +20 °C for 10 days, dark. Germinating seeds were counted and removed from the petri dishes after five, seven and 10 days at +20 °C. The seeds were counted and removed from the Petri dishes this way in order to avoid overcrowding. The total amount of germinated seeds per line was used in the QTL analyses.

AFLP linkage map

A linkage map using dominant and co-dominant AFLP-markers and a few microsatellite markers was constructed from the F_2 (Poorter et al. 2004). The map covered approximately 45% (445 cM) of the wild barley genome due to unexpectedly high heterozygosity of the Ashkelon parent. This heterozygosity was observed at first as non-segregating

F_2 sub-families, indicating that the particular F_1 parent must have been homozygous for that particular marker. These non-segregating markers within F_2 sub-families were removed from the data as described by Poorter et al. (2004). Twohundred and two (202) markers were eventually mapped on 11 linkage groups. These linkage groups were assigned to barley chromosomes based on the microsatellite and AFLP markers that were already mapped in cultivated barley. This full map was used as a basis in the QTL analysis.

Statistical analyses

Germination percentages were calculated from the germination data. Distributions of the germination percentages were non-normal and therefore arcsin square root transformations were applied (Figure 1). The QTL analysis was performed using the transformed data.

QTL analysis was conducted using MapQTL 4.0 (Van Ooijen and Maliepaard 1996). Interval mapping (IM) was used at first, multiple QTL mapping (MQM) was performed after that with inclusion of cofactors. Cofactors were selected to be markers with LOD score above 2. When the LOD values stabilised in MQM, restricted MQM (RMQM) was performed with the cofactors and these values were taken as the result of the mapping procedure. A LOD threshold value of 2.7 for a significant QTL was used based on Van Ooijen (1999). The estimated dominance effects obtained from MapQTL were multiplied by two to correct for the different generations in which marker data and phenotypic data were collected, i.e. F_2 and F_4, respectively (Verhoeven 2003).

Results and discussion

Germination percentages were calculated for each germination experiment (from day 0 to day 70). Five lines started to germinate (2.5–5%) on day 0. For a different set of five lines the onset of germination was on day 14 (2.5–7.5%). On day 28, 85% of the lines had started to germinate (2.5–75%). Only two lines remained completely dormant on day 42 and on day 56 all the lines were germinating (24–100%). Complete breaking of dormancy with 100% of germination was first observed for three lines on day 42. After 70 days of after-ripening, 42% of the lines reached germination of 90% or over. Figure 1 summarises the germination percentages from day 28 to 70.

The average germination percentages are shown in Figure 2. They are divided into three groups, i.e. (a) the total sample, (b) lines continuously increasing in germination and (c) lines decreasing in germination between days 42 and 56. The reasons for this division are explained below.

The breaking of dormancy was more or less continuously increasing in 67% of the lines (group b; Figure 2). Within these lines, the germination percentage increased or remained the same with prolonged after-ripening. However, 33% of the lines experienced a reduction in germination on

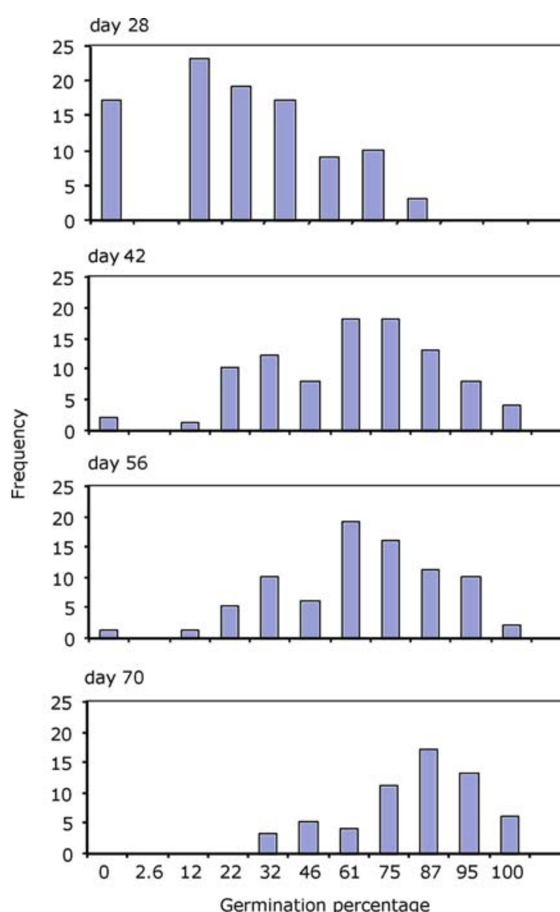

Figure 1. Frequency histograms of the transformed germination percentages (arcsin square root) of the F4 seeds in the four germination experiments used in the QTL analysis. On the *x*-axis, the germination percentages for the different classes have been back transformed.

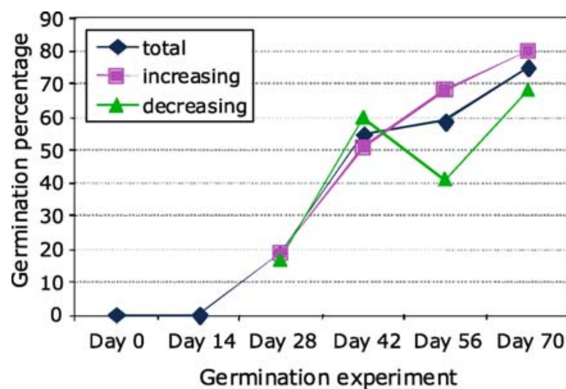

Figure 2. Average germination percentages for each germination experiment in total and divided in increasing in germination and decreasing in germination lines.

day 56 in comparison to the germination percentage of day 42 (group c; Figure 2). The most extreme line germinated only by 7.5% on day 56 after germination of 65% on day 42. On day 70, germination increased to the same level as it was on day 42 or exceeded that level substantially. Surprisingly, four lines did not seem to 'recover' but germinated less than on day 42. One of these four lines germinated even less than on day 56. Ogawara and Hayashi (1964) observed similar reduction in germination in their experiments between days 87 and 101. However, these authors did not observe such a decrease in germination in dehusked or cut seeds. This would point towards the involvement of the husk in the decrease of germination. Perhaps there is a critical after-ripening temperature and time effect on germination in nature related to survival. The seeds used in this study were intact caryopses and perhaps the hydration level of the seed coat together with the plant hormone metabolism inhibited germination of the 'decreasing' lines. This inhibitory effect, however, is mostly lost after further 14 days of after-ripening.

Another possibility is that by chance part of the seeds sampled on that day were not viable and therefore the germination percentage seemed to be decreasing. However, no selection of seeds was conducted when taking them out of the paper bags and therefore it is highly unlikely that the proportion of non-viable seeds would vary greatly among successive random samples taken from the same line, in a pattern that exactly corresponds to the observed decrease in germination. The viability of non-germinated seeds was not tested. This was assumed not to vary between the lines.

These explanations for the reduction of germination percentage on day 56 remain speculative and further research is needed to establish the reason behind these results in intact wild barley seeds. It would not be surprising, if this trend is not observed in all accessions or populations, as in the F_4 population of this study only one third of the lines showed this reduction.

Seeds from parental accessions harvested at the same time as the F_4 seeds were germinated alongside the mapping lines. Ashkelon seeds first broke dormancy on day 28 (48% germination) whereas Mehola seeds had to be after-ripened until day 70 before any germination (15%) was observed. No F_4 line was more dormant than Mehola.

There were marked differences in the rate of the release of dormancy between lines. One line lost its dormancy fast after 28 days after-ripening, gaining 100% by day 42. Another line stayed dormant having low but increasing germination percentages reaching 24% by day 70. Yet another example is a line that did not germinate until day 56 reaching 95% germination on day 70.

QTL mapping

QTL mapping was conducted on the transformed data for days 28, 42, 56 and 70. In total five QTL with LOD scores above 2.7 were detected across the experiments. The QTL were found on chromosomes 1, 2, 5, 6 and 7. Table 1 summarises the QTL results.

QTL mapping was performed at first using the full map data with 202 markers across 11 linkage groups (Poorter et al. 2004). Close inspection of the marker data revealed that 51 markers had a very low information content (less than 10 F_2 plants with known genotype). These markers were removed from the data set and thus all subsequent QTL analyses were performed using the remaining 151 markers.

The heterozygosity of the Ashkelon parent affects the QTL analysis in two ways. Firstly, we are only able to analyse 45% of the wild barley genome. Secondly, there may be three rather than two alleles segregating for any given QTL in the F_4. As a result, the estimated QTL effects will

Table 1. QTL for transformed germination data.

Exp.	Map location		LOD	% Expl.	Add. effect	Dom. effect	Nearest marker
	Chr.	cM					
day 28	2A	35	2.90	10.10	0.12	−0.08	E38M54-169
	6	14	3.46	13.3	0.10	0.20	E38M54-349
	7	12	4.57	15.4	0.15	0.04	E39M61-255
day 42	6	14	4.84	21.5	0.22	−0.02	E38M54-349
	7	12	3.39	14.6	0.17	−0.04	E39M61-255
day 56	2A	31	3.16	12.6	0.13	−0.18	E33M55-436
	5	53	2.75	11.7	−0.04	−0.38	E33M55-591
	7	11	2.11	8.6	0.12	−0.06	E33M61-221
day 70	1	42	3.95	25.0	0.20	0.08	E38M58-84

Experiment, map location, LOD values, percentage of variation explained by the QTL, additive and dominance effect of the QTL, and the associated marker. The dominance effect is corrected for F_4 as described by Verhoeven (2003).

represent the contrast between Mehola allele and the joint effect of the two Ashkelon alleles. The size of this joint effect will depend on the effect of the individual allele and its frequency in the F_4.

A QTL was detected on chromosome 1 for day 70. The significance of this QTL is fairly high (LOD 3.95) but it is based on information only from one marker class and thus is rather suspect. To find out whether there was real lack of other allele classes or whether more information could have been obtained on this occasion by using the full sample size of 233 F_2 derived F_4 lines, we checked the information content of the surrounding markers in the full sample. The results show that no more information could be gathered in this way; the nearest balanced marker remains 10 cM away from the highest LOD peak. This distortion in the marker data could be due to segregation distortion. But due to the heterozygosity of especially the Ashkelon parent, many homozygous markers per F_1 'family' were removed from the data. Therefore, little can be said about the segregation distortion in this mapping population.

Ullrich et al. (1992) detected a QTL for seed dormancy on chromosome 1 (SD3) associated with the marker *Amy*2 (position 85 cM on barley consensus map; Qi et al. 1996). Unfortunately only one of our AFLP markers on this chromosome is shared with the cultivated barley map, and therefore we cannot determine the orientation of the chromosome. Thomas et al. (1996) found two QTL on chromosome 1. One of these (near marker PBI12) maps to the same region as the SD3 QTL.

On chromosome 2A, a QTL was found for days 28 and 56 (LOD 2.90 and 3.16, respectively). The highest LOD peaks per experiment were found in the middle of the chromosome, although LOD peaks over 2.7 were also detected at the top of the chromosome and thus might point towards a possible second QTL on this chromosome. The QTL explained 10 and 12% of the variation and was consistently additive. The Per1 gene is involved in dormancy release (Stacy et al. 1996) and is nearby a marker B15C on chromosome 2 position 66 cM on the barley consensus map (Qi et al. 1996). The QTL found in this study on chromosome two is around this position. Ullrich et al. (1992) detected a QTL also near the marker B15C (ABC306, 69 cM on barley consensus map). Thomas et al. (1996) mapped two QTL on this chromosome (PBI21a, 26 cM and CDO64, 49 cM), but not around the Per1 gene.

On chromosome 5, a QTL was detected for day 56 (LOD 2.75). This was the only QTL of which the Mehola allele enhanced germination. Thus, even though Mehola is very dormant, it still contains alleles for inducing germination. The QTL explained 12% of the variation and was overdominant.

On chromosome 6 we detected a QTL for days 28 and 42. Thomas et al. (1996) detected a QTL for dormancy around this position as well. The chromosome is linked to the cultivated barley map only with one marker, so the orientation is not resolved. The QTL explained 13 and 22% of the variation for the two different days, respectively. QTL × time interaction was observed at this QTL; for day 28 the QTL was completely dominant whereas for day 42 it was additive.

On chromosome 7 a QTL was detected for days 28 and 42. A putative QTL was detected at the

same position also for day 56 (LOD 2.11). The variance explained by the QTL was about 15% for both days 28 and 42, whereas the putative QTL on day 56 explained 9% of the variation. The QTL had a consistent additive effect across the experiments. High LOD peaks were also observed on both sides of the detected QTL. These were considered to be rather suspect as markers at both ends of the chromosome are only of one marker class as was the case with the QTL on chromosome 1. Extending the population to the full set of 233 F_2 plants it appeared that no more information could be obtained for these markers. Therefore, it is not possible to determine whether there are two more QTL on this chromosome or not. To solve these problems, a new cross should be made with less heterozygous parents, so as to maximise the genome coverage.

Ullrich et al. (1992) reported a major seed dormancy QTL (SD1) on chromosome 7 explaining up to 36% of the variation in dormancy. The SD1 QTL is associated with the marker PSR128, position 76 cM on barley consensus map (Qi et al. 1996). The approximate position of the SD1 is estimated to be at the end of the chromosome on the 'Ashkelon × Mehola' map. Thus the QTL detected in this study on the same chromosome is not the SD1 QTL. It could be that the SD1 QTL is expressed also in this wild barley cross, but our map data may be failing to detect it.

Different QTL at different time points reflect a difference in the cumulative germination curves of the lines. A priori, another approach could have been taken, i.e. to fit an S-shaped curve to the observed cumulative germination data for each line and take the parameter(s) of those curves as the trait(s) to be analysed. However, because of the observed drop in germination percentage in a number of lines, this approach does not apply.

About one-third of the F_4 mapping lines experienced a reduction in germination percentage on day 56 compared to day 42. The reasons for this reduction are unknown, although based on the results of Ogawara and Hayashi (1964) the seed coverings might have something to do with the phenomenon, as this reduction in germinability seems to be restricted to intact seeds.

To establish whether QTL mapping would detect any genomic areas associated with the reduction, a new trait was defined for the reduction in germination percentage (germination percentage on day 56 – germination percentage on day 42) and QTL mapping was performed. One LOD peak of 2.32 was observed at one end of an unassigned linkage group U2 (Poorter et al. 2004) explaining 30% of the variation. A LOD value of 2.3 in this linkage group corresponds with a *p*-value of $p < 0.011$ according to a permutation test. The reduction in germination at this locus is associated with homozygosity for the Mehola allele. Individuals being homozygous for the Ashkelon allele experience less of a reduction but heterozygotes have the largest increase in germination and thus the QTL was overdominant. It is remarkable to see that the reduction in germination between day 42 and 56 is apparently being controlled by a QTL that is not involved in the 'normal' breaking of dormancy.

Conclusions

The level of dormancy is much higher in wild barley than in the crosses studied in cultivated barley. The mapping population used in this study started germinating after 28 days of after-ripening, including the less dormant parent, whereas the highly dormant parent Mehola needed 70 days of after-ripening. Removing the husk would increase the germination of wild barley seeds (Gutterman and Nevo 1994), but we doubt whether this would be enough to allow germination of Mehola seeds immediately after harvest.

We have detected several QTL for breaking of seed dormancy in a wild barley cross. Because the 'Ashkelon × Mehola' map does not have enough markers linking it to the cultivated barley map, the QTL found here could not be fully compared with the QTL found earlier with confidence. However, some comparisons could be made revealing that three out of the five detected QTL map to the same regions with QTL found earlier in cultivated barley crosses. These wild barley QTL could be the same as the ones detected in cultivated barley, but it must be noted that they can also be different loci situated near each other. Thus, wild barley may harbour dormancy controlling genes that have not yet been exploited by barley breeders.

This available variation in dormancy release in wild barley could be further investigated through crossing wild barley accessions with diverse dormancy levels with cultivated barley varieties.

Crosses between a cultivar and Ashkelon or Mehola would further clarify the QTL detected in this study. Association mapping, on the other hand, using the whole range of wild *Hordeum spontaneum* accessions and mapped markers would provide an interesting and useful inventory of the presence of dormancy QTL and genes in the populations across the whole of Israel.

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