Theor Appl Genet (2005) 110: 445–453
DOI 10.1007/s00122-004-1851-1

ORIGINAL PAPER

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# Genetic basis of barley caryopsis dormancy and seedling desiccation tolerance at the germination stage

Received: 20 July 2004 / Accepted: 18 October 2004 / Published online: 24 November 2004


**Abstract** The genomic regions controlling caryopsis dormancy and seedling desiccation tolerance were identified using 152 $F_4$ lines derived from a cross between Mona, a Swedish cultivar, and an Israeli xeric wild barley *Hordeum spontaneum* genotype collected at Wadi Qilt, Israel. Dormancy, the inability of a viable seed to germinate, and desiccation tolerance, the ability of the desiccated seedlings to revive after rehydration, were characterized by fitting the germination and revival data with growth curves, using three parameters: minimum, maximum, and slope of germination or revival rate derived by the least square method. The genetic map was constructed with 85 genetic markers (SSRs, AFLPs, STSs, and Dhn genes) using the MULTIPOINT-mapping algorithm. Quantitative trait loci (QTLs) mapping was conducted with the MULTIQTL package. Ten genomic regions were detected that affected the target traits, seven of which affected both dormancy and desiccation tolerance traits. Both the wild barley genotype and the Swedish cultivar contributed the favorite alleles for caryopsis dormancy, whereas seedling desiccation tolerance was attributed to alleles descending from the cultivar. The results indicate that some barley dormancy genes are lost during domestication and that dormancy QTLs are associated with abiotic stress tolerance.

Communicated by R. Hagemann

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## Introduction

Seed dormancy is defined as the inability of a viable mature seed to germinate under conditions deemed to be adequate for germination (Simpson 1990). As this trait currently cannot be measured directly, seed germination percentage is the parameter usually used to measure it. Under natural conditions, the primary dormancy of barley is an important survival strategy in that it regulates the proper time for dormancy release and the relationship between the amount of rain and percentage of dormancy release during the winter following maturation (Evenari and Gutterman 1976). Caryopsis dormancy allows plants to escape drought and high temperatures in the warmer summer months (Snape et al. 2001). For cultivated barley, a balance between a limited level of dormancy at grain maturity and rapid germination after seed harvest is highly warranted (Prada et al. 2004).

Genetic control of barley seed dormancy has been studied by means of quantitative trait loci (QTLs) mapping. Four regions on chromosomes 4, 5, and 7H have been associated with most of the differential genotypic expression for dormancy in the Steptoe/Morex (S/M) mapping population (Ullrich et al. 1993; Han et al. 1996; Larson et al. 1996), while two regions on chromosomes 1 and 5H that control dormancy have been identified with the Harrington/TR306 (H/T) population (Takeda 1996), and four genomic regions have been identified on chromosomes 2, 3, and 5H for dormancy in the Triumph/Morex (Tri/M) population (Prada et al. 2004). Edney and Mather (2004) identified four regions that affected germination (4 ml water) on chromosomes 2, 5, and 7H in the Harrington/Morex (H/M) population.

In addition to dormancy, post-germination seedling desiccation tolerance is one of the strategies adopted by wild barley to cope with problems caused by the scarcity and unpredictability of precipitation events in the xeric steppes and deserts (Evenari et al. 1971; Gutterman 1993; Blondel and Aronson 1999). The tolerance of wild barley seedlings to desiccation varies between genotypes from different habitats (Gutterman and Gozlan 1998; Zhang et al. 2002; Chen et al. 2004).

Verhoeven et al. (2004) studied the genetic basis of the desiccation tolerance of wild barley seedlings in an $F_4$ mapping population derived from a cross between two wild barley genotypes but was unable to identify a significant QTL effect. With respect to tolerance to other abiotic stresses at the germination stage in barley, Mano and Takeda (1997) identified two and three salt tolerance QTLs in the H/T and S/M mapping populations, respectively.

The objective of the investigation reported here was to identify chromosome regions controlling caryopsis dormancy and seedling desiccation tolerance of barley by employing the powerful multiple trait QTL analysis (Korol et al. 2001) combined with multiple interval mapping (MIM) (Kao et al. 1999).

## Materials and methods

### Plant materials

Wild barley (*Hordeum spontaneum* C. Koch) Wadi Qilt genotype 23-39, originated in a desert population from Israel (140 mm annual rainfall), was crossed to *H. vulgare* cv. Mona (Swedish cultivar). A mapping population of 152 $F_4$ families was established for QTL mapping. The $F_3$ families were grown in an open field at the Sede Boker Campus in the northern Negev Desert, Israel, under conditions of high-water availability (approx. 400 mm annual rainfall) and low-water availability (approx. 200 mm annual rainfall), respectively. Therefore, $F_4$ mapping lines included two groups in terms of the amount of water their respective mother plants had received.

### Test of dormancy and desiccation tolerance

Caryopses (6×100) of each line of $F_4$ individuals were moistened with 4 ml distilled water in 90-mm Petri dishes on one piece of Whatman No. 1 filter paper and incubated in the dark for 21 days. During these 3 weeks of incubation, the moistened caryopses were kept at 20°C during the first and the last week and at 5°C during the second week. Germination was checked at 3, 5, 7, 10, 14, 17, and 21 days of the incubation period, and the germinating caryopses were removed and subsequently dried under laboratory conditions by being placed on filter paper in open Petri dishes (relative humidity: 0–10%, temperature: 25–30°C) for 4 weeks. The seedlings were subsequently re-moistened and moved to a dark chamber at 15°C for 5 days. On the fifth day, the revival rate was observed by checking re-growth and the appearance of adventitious roots (Ashby and May 1941).

### Traits for QTL mapping analysis

The dynamic process of the germination rate and revival rate at designated times was fitted by a growth curve (the logistic growth function) characterized by a genotype-specific minimum value ($X^0{}_g$), maximum value ($X^m{}_g$), and slope parameter ($S_g$),

$$X_g(t) = \frac{X_g^m}{1 + (X_g^m/X_g^0 - 1)\exp\left[-S_g(t - t_0)\right]},$$

where $t_0$ and $t$ are the initial and current days of measurements (Wu et al. 2004). The fitting of the curve was made by searching for the parameter values that minimize the square of distances between the observed and approximated scores. Twelve traits—GCmin, GCmax, GCs, GDmin, GDmax, GDs, RCmin, RCmax, RCs, RDmin, RDmax, and RDs—representing the minimum (min), maximum (max), and slope parameter (s) of the germination rate (G) and the revival rate (R) of caryopses derived from control (C) and drought-stressed (D) $F_4$ lines—were mapped.

### Genomic DNA extraction and genotyping

Genomic DNA of $F_4$ families representing $F_2$ individual plants was extracted using the plant genomic DNA isolation reagent DNAzol ES (Molecular Research Center, Cincinnati, Ohio), with some modifications (Peng et al. 1999). Genotyping with simple sequence repeat (SSR) markers (Ramsay et al. 2000) was according to the method described by Peng et al. (1999). Genotyping with amplified fragment length polymorphism (AFLP) markers was according to Vos et al. (1995). Genotyping with sequence-tagged site (STS) markers was according to Talbert et al. (1994). Genotyping with dehydrin (Dhn) gene markers (Choi et al. 1999) was conducted by means of PCR analyses. Approximately 100–150 ng of template DNA, 250 n $M$ of each primer, 200 $\mu M$ of dNTPs, 0.8 U *Taq* polymerase and 1.5 m $M$ $MgCl_2$ were mixed in a reaction mix and subjected to 45 cycles of amplification (denaturing at 94°C for 1 min; annealing at 55°, 60°, or 65°C, depending on the primer pair, for 1 min; extension at 72°C for 2 min), followed by a final extension step at 72°C for 10 min and end at 10°C. The amplification products were detected on 2% agarose gels using a GibcoBRL (Gaithersburg, Md.) electrophoresis unit. The fragments separated on the agarose gel were stained with ethidium bromide and

the results digitalized using a black and white camera under UV illumination.

### Genetic linkage map and QTL mapping

Molecular markers, including 48 SSRs, four STSs, two Dhn genes, and 31 AFLPs, were used to construct a 2,058-cM genetic linkage map based on the evolutionary strategy algorithm implemented in the MULTIPOINT mapping software (Mester et al. 2003). The map distances in the framework maps were computed using the Haldane mapping function (Lincoln et al. 1992). The software used in the QTL analysis was the MULTIQTL package (http://esti.haifa.ac.il/~poptheor/MultiQtl/MultiQtl.htm). Each chromosome was dissected into two or three segments due to big gaps. These segments were used separately as linkage groups for QTL mapping, for which we used four approaches: (1) single-trait analysis, (2) MIM-single-trait analysis, (3) multiple-trait analysis, and (4) MIM-multiple-trait analysis. Two QTL effects were estimated: the substitution effect, $D = 2d = (X_{QQ} - X_{qq})$, and the heterozygous effect, $h = X_{Qq} - \mu$, both at a QTL $Q/q$ for trait $X$.

## Results

### Trait distributions and correlations

A large variation was observed in the progeny for all traits with a coefficient of variation (CV) ranging from 34.1% to 99.6% (Table 1). The distributions of all traits showed strong transgressive segregations, suggesting that the Mona genotype also contained positive alleles for the traits. The traits correlated to one another, and all of the significant correlations were positive (Table 2). The significant positive correlations can be seen in the germination rate versus revival rate scores in the control versus drought-stressed plants, and in the maximum versus minimum scores. The four slope parameters (GCs, GDs, RCs, and

**Table 1** Trait values of the two parents and 152 $F_4$ lines

| Trait[a] | $F_4$ lines | | | | Mean: 23-39 | Mean: Mona |
|---|---|---|---|---|---|---|
| | Mean | Minimum | Maximum | Standard deviation | | |
| GCmax | 62.5 | 10.4 | 98.1 | 21.3 | 36.2 ± 15.6 | 96.1 ± 4.4 |
| GCmin | 28.8 | 0.1 | 94.3 | 23.8 | 1.4 ± 2.1 | 89.3 ± 16.7 |
| GCs | 0.5 | 0.1 | 3.5 | 0.5 | 0.4 ± 0.2 | 0.6 ± 0.4 |
| GDmax | 59.9 | 5.0 | 100.0 | 23.2 | 30.8 ± 15.9 | 98.9 ± 2.8 |
| GDmin | 29.5 | 0.1 | 95.7 | 24.9 | 1.0 ± 1.8 | 96.6 ± 6.4 |
| GDs | 0.6 | 0.1 | 2.3 | 0.4 | 0.5 ± 0.2 | 0.6 ± 0.5 |
| RCmax | 33.1 | 2.7 | 78.3 | 17.8 | 15.7 ± 8.9 | 66.8 ± 9.5 |
| RCmin | 15.9 | 0.0 | 62.7 | 15.0 | 0.8 ± 1.4 | 64.8 ± 11.4 |
| RCs | 0.7 | 0.1 | 4.4 | 0.6 | 0.5 ± 0.2 | 0.1 ± 0.1 |
| RDmax | 32.0 | 0.7 | 82.3 | 18.6 | 14.9 ± 9.9 | 57.8 ± 22.2 |
| RDmin | 16.5 | 0.0 | 76.0 | 16.4 | 0.6 ± 1.0 | 57.6 ± 22.1 |
| RDs | 0.7 | 0.1 | 3.4 | 0.6 | 0.5 ± 0.2 | 0.2 ± 0.3 |

[a]GCmin, GCmax, GCs, GDmin, GDmax, GDs, RCmin, RCmax, RCr, RDmin, RDmax, and RD, represent minimum (min), maximum (max), and slope (s) of germination rate (G) and revival rate (R) of caryopses derived from control (C) and drought-stressed (D) $F_4$ lines, respectively

**Table 2** Correlations among traits based on the 152 $F_4$ lines

| | GCmax[a] | GCmin | GCs | GDmax | GDmin | GDs | RCmax | RCmin | RCs | Rdmax | RDmin |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GCmin[a] | 0.70*** | | | | | | | | | | |
| GCs | 0.12 | 0.11 | | | | | | | | | |
| Gdmax | 0.68*** | 0.57*** | 0.13 | | | | | | | | |
| GDmin | 0.54*** | 0.81*** | 0.16 | 0.74*** | | | | | | | |
| GDs | 0.13 | 0.15 | 0.64*** | 0.18* | 0.21* | | | | | | |
| RCmax | 0.75*** | 0.41*** | 0.15 | 0.59*** | 0.37*** | 0.13 | | | | | |
| RCmin | 0.65*** | 0.81*** | 0.16 | 0.60*** | 0.71*** | 0.22** | 0.73*** | | | | |
| RCs | −0.01 | 0.02 | 0.79*** | 0.03 | 0.09 | 0.46*** | −0.03 | −0.02 | | | |
| RDmax | 0.57*** | 0.39*** | 0.10 | 0.82*** | 0.55*** | 0.17* | 0.76*** | 0.66*** | −0.02 | | |
| RDmin | 0.48*** | 0.63*** | 0.19* | 0.71*** | 0.84*** | 0.27** | 0.57*** | 0.80*** | 0.07 | 0.79*** | |
| RDs | 0.14 | 0.09 | 0.42*** | 0.12 | 0.15 | 0.67*** | 0.12 | 0.14 | 0.47*** | 0.13 | 0.16 |

Levels of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

[a]GCmin, GCmax, GCs, GDmin, GDmax, GDs, RCmin, RCmax, RCr, RDmin, RDmax, and RDs represent minimum (min), maximum (max) and slope (s) of germination rate (G) and revival rate (R) of caryopses derived from control (C) and drought-stressed (D) $F_4$ lines, respectively

**Table 3** QTLs detected by single-trait analysis with the single-QTL/single-chromosome model

| Trait[a] | Chromosome | *P* value | Power | LOD | Length (centiMogans) | PEV[b] | Selected sub-model[c] | Substitution effect | Heterozygous effect |
|---|---|---|---|---|---|---|---|---|---|
| Gcmax | $1H_1$ | 0.0285 | 37 | 3.24 | 27.1 ± 5.8 | 13.1 ± 4.5 | Het | – | −59.2 ± 10.7 |
| Gcmax | $5H_2$ | 0.0145 | 51 | 3.58 | 64.5 ± 18.4 | 14.1 ± 4.5 | Dom | 19.9 ± 3.8 | 10.0 ± 1.9 |
| GCmin | $1H_2$ | 0.0142 | 49 | 8.03 | 36.5 ± 8.3 | 44.8 ± 9.9 | Dom | −24.5 ± 2.9 | −13.2 ± 2.4 |
| Gdmax | $7H_1$ | 0.0167 | 55 | 3.78 | 84.4 ± 8.9 | 12.5 ± 3.8 | Het | – | −61.6 ± 18.8 |
| Gdmax | 7H2 | 0.0163 | 51 | 4.19 | 54.1 ± 11.8 | 39.0 ± 11.5 | Het | – | −120.0 ± 20.1 |
| GDmin | 1H2 | 0.0448 | 35 | 7.25 | 38 ± 5.1 | 49.5 ± 7.6 | Gen | −37.1 ± 7.9 | −124.0 ± 15.6 |
| Rcmax | $5H_1$ | 0.0053 | 56 | 3.48 | 87.2 ± 6.5 | 11.7 ± 3.6 | Rec | −15.9 ± 3.1 | 8.0 ± 1.5 |
| RCs | 4H | 0.0215 | 27 | 15.4 | 9.8 ± 8.3 | 64.0 ± 5.1 | Gen | −1.4 ± 0.2 | −3.2 ± 0.4 |
| Rdmax | 5H1 | 0.0217 | 37 | 3.18 | 86.4 ± 7.7 | 11.1 ± 3.2 | Add | −17.2 ± 2.9 | – |
| Rdmax | 7H1 | 0.0216 | 42 | 3.23 | 2.4 ± 13.8 | 10.6 ± 2.6 | Add | −15.6 ± 5.1 | – |
| Rdmax | 7H2 | 0.0195 | 40 | 3.59 | 56.8 ± 11.4 | 25.2 ± 8.6 | Het | – | −76.2 ± 12.2 |
| RDmin | 2H | 0.0408 | 30 | 9.02 | 3.1 ± 3.4 | 66.9 ± 5.8 | Het | – | 125.0 ± 10.3 |
| RDmin | 3H | 0.0050 | 61 | 4.14 | 4.4 ± 9.1 | 17.2 ± 13.5 | Het | – | 55.1 ± 27.5 |
| RDmin | 7H1 | 0.0297 | 37 | 3.29 | 2.3 ± 12.4 | 11.0 ± 3.2 | Dom | −13.9 ± 4.4 | −7.0 ± 2.2 |
| RDs | 4H | 0.0004 | 59 | 19.2 | 6.1 ± 4.1 | 68.3 ± 5.2 | Gen | −1.6 ± 0.2 | −4.2 ± 0.4 |

[a]GCmin, GCmax, GCs, GDmin, GDmax, GDs, RCmin, RCmax, RCr, RDmin, RDmax, and RDs represent minimum (min), maximum (max) and slope (s) of germination rate (G) and revival rate (R) curves of caryopses derived from control (C) and drought-stressed (D) $F_3$ lines.

[b]PEV is the percentage of explained trait variation

[c]Dom, dominant; Add, additive; Het, heterotic; Gen, general; Rec, recessive

RDs) were significantly correlated to each other but not to other traits.

## QTLs identified

Single-trait analysis using the single-chromosome/single-QTL model revealed 15 QTL effects ($P < 0.05$) (Table 3) that were located on all chromosomes except 6H (Fig. 1). Although these QTL effects were not significant when the false discovery rate (FDR) was controlled at 10% and one QTL effect (RDs on chromosome 4H) was significant when the FDR was set at 20%, these QTL effects were confirmed by collocation with QTL effects identified by the same analysis or another analysis such as MIM-single-trait, multiple-trait, and MIM-multiple-trait analysis (Fig. 1). Therefore, these QTL effects were relevant and worth further investigation.

In five chromosome regions, a QTL effect was collocated with a second QTL effect; for example, a QTL effect for RDmax was collocated with a QTL effect for RDmin in the region $CD7H_1$. In the other five chromosome regions, there was one QTL effect per region identified by single-trait analysis. In these regions a QTL effect was collocated with one or several other QTL effects identified by MIM-single-trait analyses. The QTL effects identified by MIM-single-trait analyses were more significant and accurate than those identified by the single-trait analyses. For instance, in region $CD5H_1$ for RCmax, the QTL effects identified by MIM-single-trait analyses and single-trait analyses had $P = 0.0000$ and $P = 0.0053$, a positional standard deviation of 3.4 cM and 6.5 cM, respectively (Fig. 2a); in region CD5H2 for GCmax, the QTL effects identified by MIM-single-trait analyses and single-trait analyses had $P = 0.0018$ and 0.015, a positional standard deviation of 10.9 cM and 18.4 cM, respectively.

Multiple-trait analyses and MIM-multiple-trait analyses identified two chromosomal regions ($CD1H_2$ and $CD4H_1$ controlling caryopsis dormancy and seedling desiccation tolerance) and confirmed the QTL effects identified by single-trait analyses. For example, the QTLs identified by multiple-trait analyses and MIM-multiple-trait analyses in region $CD1H_2$ had a similar significant level ($P < 0.001$) and similar effects on GCmax, GCmin, GDmin, and RCmin (data not shown); they confirmed the QTL effects for GCmin and GDmin identified by the single-trait analyses and revealed two new QTL effects for GCmax and RCmin in this region (Fig. 2b).

QTLs that affected GCmax were located on chromosome segment $1H_1$ and $5H_2$,while QTLs that affected

**Fig. 1** Genomic regions controlling barley caryopsis dormancy and seedling desiccation tolerance. Chromosome segments that harbor QTLs are shown with the interval distances calculated using the Haldane mapping function. *GCmin*, *GCmax*, *GCs*, *GDmin*, *GDmax*, *GDs*, *RCmin*, *RCmax*, *RCs*, *RDmin*, *RDmax*, and *RDs* represent minimum (*min*), maximum (*max*), and slope (*s*) of germination rate (*G*) and revival rate (*R*) of caryopses derived from control (*C*) and drought-stressed (*D*) $F_3$ lines. QTLs identified by single-trait and MIM-single-trait analysis are indicated by the *trait name*. QTLs detected by multi-trait and MIM-multi-trait analysis are indicated with the *multi-trait name followed by traits* that were significantly affected by the multi-trait QTLs. *GR* multi-trait names for the trait group containing all the germination and revival traits, *GRD* the trait group containing the germination and revival traits derived from drought-stressed mother plants, *RC*, *RD* for the trait group containing revival traits derived from non-stressed and drought-stressed mother plants, respectively. *Bar* indicates the QTL location; the *length* is equal to double standard deviation. The short straight lines next to bars indicate genomic regions

Single-trait
MIM-single-trait
Multiple-trait
MIM-multiple-trait
Dissected interval
Genomic region
CD5H1
RCmax
RCmax
RDmax
CD5H2
GCmax
RDs
GCmax
5H
GMS61-1
EBMAC824-4
GMS27-3
E35M55kx-1
BMAG222-1
E38M55no-2
BMAG223-4
MWG583-3
BMAG812-1
E35M59mg-4
DHN1-3
20.5
38.4
20.8
29.2
16.3
14.0
17.5
40.8
45.1
CD7H1
RDmin
RDmax
CD7H2
GDmin
GDmax
CD7H3
RCmax
RDmax
RDmax
GDmax
7H
EMAG794A-3
BMAG7-3
AF22725-3
BMAG341A-2
BMAG507-2
EMAG757B-3
HVM11-4
BMAG135-4
HVPR1B-2
HVM49-2
12.6
49.8
42
7.8
24.9
26.2
35.7
35.7
CD1H1
RD:RDs
GDmin
GCmin
RDs
GCmax
CD1H2
GR:GCmin,GCmax,RCmin,GDmin
GR:GCmin,GCmax,RCmin,GDmin
GCmin
GDmin
CD2H1
GDmin
RDmin
CD3H1
GDmin
RDs
RDmax
RDmin
CD4H1
GRD:GDmin,RDs
RD:RDs
GR:GDmin,GDmax,RDmin
RCD:RDmin,RDs
RCs
RDs
RDs
RCs
1H
EE38M48pa-4
ABC160-3
BMAC32-4
HVM20-2
BMAG211-3
EMBAC659-3
EE38M55ob-1
EE35M59lq-5
27.8
19.4
5.9
19.7
16.7
17.7
2H
E38M48ou-5
E35M59lt-5
EBMAG793-1
29.7
43.6
3H
EBMAC541-1
E35M55ke-4
BMAG13-3
ABGO04-3
31.6
23.1
16.3
4H
E33M54ax-5
BMAG303A-3
E35M55kq-5
HVM3-3
HVOLE-3
HVM40-3
19.1
13.6
14.8
43.6
10.9

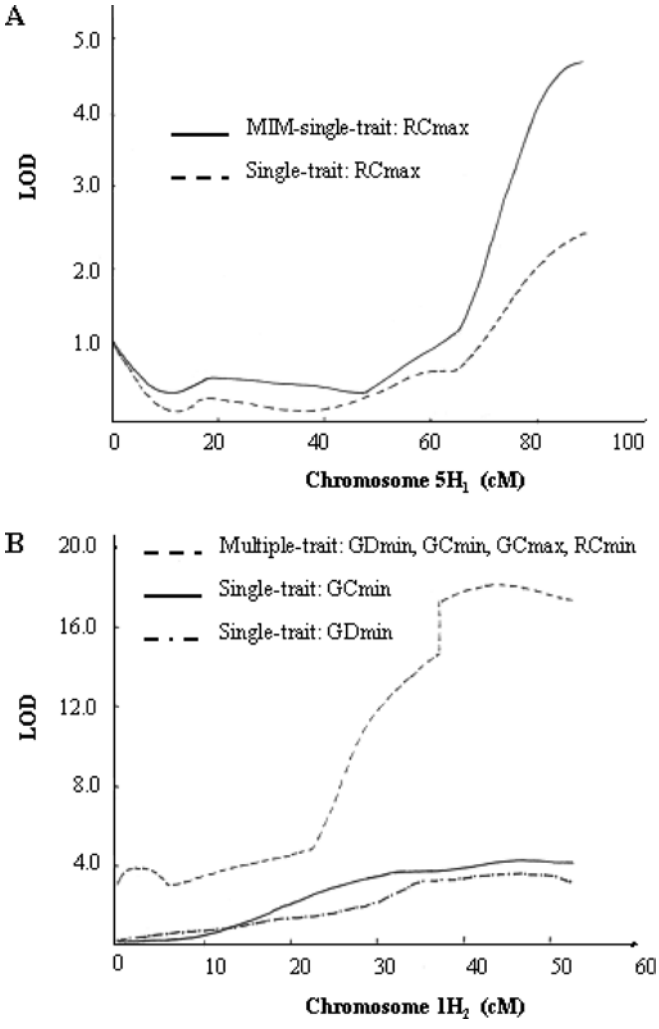


**Fig. 2** Chromosome locations and LOD scores on $5H_1$ of the QTL effects for RCmax identified by single-trait and MIM-single-trait analyses (**a**) and on $1H_2$ of the QTL effects for GCmin and GDmin identified by single- and multiple-trait analyses (**b**)

GDmax were located on chromosome segments $7H_1$ and $7H_2$ (Table 3). The GDmax QTL on chromosome segment $7H_2$ explained 39.0% of the phenotypic variation, while the other three GCmax and GDmax QTLs explained about 13% of the phenotypic variation. Wadi Qilt alleles for GCmin and GDmin QTLs on chromosome segment $1H_2$ had negative substitution effects, which indicates that they reduce the minimum germination rate and are, thereby, responsible for enhancing caryopsis dormancy, whereas that for GCmax QTL on chromosome segment $5H_2$ acted in the opposite direction. QTLs for GCmax on chromosome segment $1H_1$ and GDmax on chromosome segments $7H_1$ and $7H_2$ had heterotic effects, which indicate that Wadi Qilt alleles affected caryopsis dormancy via an interaction with Mona alleles. GCmin and GDmin QTLs were located at the same position on chromosome segment $1H_2$ and revealed the same negative effects from the Wadi Qilt allele, explaining about 44.8–49.5% of the phenotypic variation. With respect to seedling revival rate, more QTLs were detected for seedlings derived from the seeds harvested from drought-stressed plants than from those harvested from non-stressed plants. RCs and RDs QTLs were located at the same position on chromosome 4H with similar effects. Similarly, we found QTLs for RCmax and RDmax on chromosome segment $5H_1$, for RDmax and RDmin on chromosome segment $7H_1$, and for RDmax and GDmax on chromosome segment $7H_2$. Among the nine QTLs for seedling revival rate, Wadi Qilt alleles for six QTLs reduced the value of the trait, while the other three QTLs affected the trait via an interaction with Mona alleles. QTLs for RDs and RCs on chromosome 4H, RDmin on chromosome 2H explained 68.3%, 64.0%, and 66.9% of the phenotypic variation, respectively. QTLs for RDmax on chromosome segment $7H_2$ and RDmin on chromosome segment $3H_2$ explained 25.2% and 17.2% of the variation, respectively. The other four QTLs each explained about 11% of the variation.

## Discussion

### Caryopsis dormancy and seedling desiccation tolerance

The minimum, maximum, and slope of germination and of the revival rate of caryopses derived from drought-stressed mother plants were compared with those derived from non-drought-stressed mother plants. There was no significant difference between these traits in the $F_4$ mapping populations and in both parents (Table 1), indicating that caryopsis dormancy and seedling desiccation tolerance were not affected by drought stress on the mother plants. This is in agreement with the high correlations observed between the traits measured from the lines derived from drought-stressed and non-stressed mother plants (Table 2) and is probably due to the overlapping QTLs for the traits measured from the lines derived from drought-stressed and non-stressed mother plants (Table 3, Fig. 1).

For both drought-stress-derived and non-stress-derived caryopses, the germination rate—either minimum or maximum—was significantly correlated with the revival rate. The slope of the germination rate was also significantly correlated with the slope of the revival rate, irrespective of whether the caryopses were drought-stress-derived or non-stress-derived (Table 2). These results indicate that caryopsis dormancy is correlated with seedling desiccation tolerance, which may be explained by the seven-out-of-ten identified genomic regions displaying both dormancy and desiccation tolerance QTL effects (Fig. 1). Although no comparable results have been reported in the literature, indirect evidence has been found to support the relationship of caryopsis dormancy and seedling desiccation tolerance. Plants occurring in the more extreme environmental conditions produce many more dormant seeds than plants occurring in less extreme

conditions (Baskin and Baskin 1998). Verhoeven et al. (2004) investigated seedling desiccation tolerance of the recombinant progeny of a cross between a plant with big seeds (from a coastal *H. spontaneum* population near Ashqelon, Israel; 424 mm of annual rainfall) and a plant with small seeds (from an inland population near Mehola, Israel; 270 mm of annual rainfall). It was assumed that deeper dormancy occurred in the small seeds from more extremely adapted Mehola population than in the large seeds from the less extremely adapted Ashqelon one. The large-seeded Ashqelon parent showed a better survival after desiccation than the small-seeded Mehola parent, and this positive association between seed mass and desiccation tolerance was maintained in the $F_4$ progeny (Verhoeven et al. 2004). One may infer from these results that plants with less dormant large seeds have a stronger seedling desiccation tolerance, which is in agreement with the negative association between dormancy and seedling desiccation tolerance found in the present study.

## Collocations of the present and known QTLs for dormancy

Eight chromosome regions controlling caryopsis dormancy were detected in the investigation reported here (Fig. 1). Three regions were new and five were consistent with regions known to control seed dormancy and germination on the basis of previous studies. Chromosome regions CD3H and CD4H coincide with a dormancy QTL (Prada et al. 2004) and a seed dormancy QTL4 (SD4) (Han et al. 1996), respectively. Chromosome region CD5H1 coincides with SD1 (Han et al. 1996), a dormancy QTL (Prada et al. 2004), and a germination QTL (Edney and Mather 2004). Chromosome region $CD5H_2$ coincides with a seed dormancy QTL (SD2) (Han et al. 1996), a dormancy QTL (Prada et al. 2004), and a dormancy QTL detected by Takeda (1996). Chromosome region CD7H1 coincides with germination QTL (Edney and Mather 2004). Four regions (SD1–4) were associated with most of the differential genotypic expression for dormancy in the S/M mapping population (Ullrich et al. 1993, 1995; Han et al. 1996; Larson et al. 1996). Three of these have corresponding regions in the present study. Takeda (1996), with the H/T population, identified two regions that controlled dormancy, one of which corresponds to a region in the present study. Four genomic regions were identified to control dormancy with the Tri/M population (Prada et al. 2004); three of these have corresponding regions in the present study. Edney and Mather (2004) identified four regions that affected normal germination (4 ml water per dish) with the H/M population; two of these have corresponding regions in the present study. In general, a large number of genomic regions are detected using a mapping population derived from a cross of wild barley with a cultivar than from a cross of cultivar with another cultivar. More than one-half of the dormancy regions in cultivar genomes have corresponding regions in the wild barley genome. The cultivar dormancy regions without corresponding regions in the present study may be identified in other wild barley crosses. Therefore, we conclude that there are many dormancy genes in wild barley, some of which were lost during domestication and breeding. Cultivars of different pedigrees may maintain different dormancy genes at different loci, which may explain why mapping populations derived from different cultivars did not reveal the same QTLs.

## Abiotic stress tolerance and dormancy QTLs

Using a cross of two wild barley genotypes, Verhoeven et al. (2004) attempted to map QTLs that control post-germination seedling survival rates after a 6-day-long desiccation in $F_4$ progeny families but failed to identify a significant QTL effect. Mano and Takeda (1997) report the mapping of QTLs for abiotic stress tolerance at the germination stage in barley: two and three salt tolerance QTLs were identified in the H/T and S/M mapping populations, respectively. The salt tolerance QTLs on chromosomes 1 and 5H in the H/T cross and on chromosome 4H in the S/M cross might coincide with the $CD1H_2$, $CD5H_1$, and CD4H regions in the present study where these three regions were associated with caryopsis dormancy and post-germination seedling desiccation tolerance. Both salt tolerance and desiccation tolerance at the germination stage are associated with seed dormancy by sharing the same genomic regions. Actually, seed dormancy of wild barley is a characteristic of an abiotic stress tolerance because in nature seed dormancy allows plants to escape drought and high temperatures in the warmer summer months (Snape et al. 2001). One may infer that dormancy QTLs are associated with those QTLs that control abiotic stress tolerance. Six of the ten dormancy QTLs identified in the present study are coincident with or near the abiotic stress tolerance QTLs: CD1H2 with a salt and drought tolerance QTL on chromosome 1H; CD2H with a drought tolerance QTL on chromosome 2H; CD4H with a salt tolerance QTL on chromosome 4H; CD5H1 and CD5H2 with a salt tolerance and a cold tolerance QTL, respectively; CD7H3 with a drought tolerance QTL (Cattivelli et al. 2002). The coincidence of dormancy QTLs with the abiotic stress tolerance QTLs indicated that there might be a common physiological basis for dormancy, seedling desiccation, salt, drought, or cold tolerance. Drought, salinity, and freezing are stresses which all lead to cellular dehydration through different mechanisms. This common component of water stress is evident in shared molecular responses to these stresses. Examples of such responses are common genes induced by all three types of stress and the importance played by the phytohormone abscisic acid (ABA).

ABA also regulates the signaling elements that mediate the regulation of seed dormancy and germination (Leung and Giraudat 1998).

## QTL effects on seedling desiccation tolerance

We attributed nine desiccation tolerance QTL effects to wild barley alleles (Table 3). Six of the substitution effects of these wild barley alleles were negative, which indicates that the wild barley alleles decreased the desiccation tolerance of the seedling; in other words, for these loci, Mona contributed the favorite alleles for seedling desiccation tolerance. This may be the genetic basis for our finding that Mona showed a higher revival rate than wild barley (Table 1). Seven heterozygous effects of QTLs for desiccation tolerance were detected; three were positive and four were negative (Table 3). Positive heterozygous effects indicate that the desiccation tolerance of heterozygotes was higher than the mean of the parents, while negative heterozygous effects indicate that the desiccation tolerance of the heterozygotes was lower than the mean of the parents. The values of QTL heterozygous effects for seedling desiccation tolerance show that the positive and negative heterozygous effects counteracted each other. Therefore, QTL effects for seedling desiccation tolerance were revealed by additive effects.

Mona and the $F_4$ progenies containing Mona alleles exhibited a higher desiccation tolerance at the germination stage, perhaps because Mona alleles retain the function of desiccation tolerance while losing the dormancy function (discussed in the preceding section). It may also be explained by the difference in seed size. Mona seeds are larger than wild barley seeds (data not shown). In an $F_4$ mapping population derived from a cross between two wild barley genotypes, seed size was positively correlated with post-germination seedling survival rates following a 6-day-long desiccation (Verhoeven et al. 2004). In germinating barley seeds, water stored in the endosperm retracts towards the embryo during desiccation, thereby reducing both the rate and the degree of dehydration stress in the developing embryo (Allen et al. 2000). This suggests that a larger seed may confer better adaptive protection against temporary drought to the embryo or to the basal meristematic tissue.

In conclusion, caryopsis dormancy and seedling desiccation tolerance were not affected by drought stress on the mother plants but rather were correlated to each other; dormancy QTLs were associated with abiotic stress tolerance; some wild barley dormancy genes were lost during domestication.

**Acknowledgements** This work was supported by the U.S. AID Cooperative Development Research Program (grant TA-MOU-97_CA17-001), German-Israeli Project Cooperation (grant DIP-B-4.3), the Israel Discount Bank Chair of Evolutionary Biology, and the Ancell-Teicher Research Foundation for Molecular Genetics and Evolution.

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