

Chromosomal regions controlling seedling drought resistance in Israeli wild barley, *Hordeum spontaneum*

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Abstract The objective of the present study was to map quantitative trait loci (QTL) controlling drought-resistance traits at seedling stage of Israeli wild barley (*Hordeum spontaneum*). Mapping was conducted using an F₄ mapping population derived from a cross between genotype WQ23-38 from xeric (Wadi Qilt, 144 mm annual rainfall) and MA10-30 from mesic (Maalot, 790 mm annual rainfall) environments. Obvious segregation was observed for traits including time to wilt, leaf relative elongation rate, recovery rate, shoot regrowth, osmolarity, relative water content, leaf length, and root length with the coefficient of variation ranging from 10 to 77%. Eighteen putative QTL effects were identified (at false discovery rate = 10%) by single-trait analyses and confirmed by multi-trait analysis and

multiple interval mapping using a new MultiQTL package. The results of our study suggest that the xeric genotypes adopted survival strategies while the mesic genotypes adopted growth-sustain strategies to cope with drought stress.

Keywords Drought resistance · Genetic map · *Hordeum spontaneum* · QTL mapping · Wild barley

Introduction

Among the different abiotic stresses, drought is by far the most complex and devastating on a global scale (Pennisi 2008). The sustainable and economically viable solution for increasing crop productivity in arid and semiarid zones is genetic improvement (Blum 1988; Stanhill 1997). It is often discussed and emphasized that crop genetic improvement lies in exploiting the gene pools of the wild relatives of the crop plant (Feldman and Sears 1981; Nevo 1995; Nevo et al. 2002). We anticipate a growing interest in wild relatives of crops and landraces in an attempt to identify superior alleles among those that the domestication bottleneck and modern agriculture have left behind (Tanksley and McCouch 1997; Grandillo et al. 2007; Lippman et al. 2007; Feuillet et al. 2008).

Wild barley, *H. spontaneum*, the progenitor of cultivated barley, is a selfing annual grass of

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predominantly Mediterranean and Irano–Turanian distribution that penetrates into desert environments where it maintains stable populations (Harlan and Zohary 1966). The wide ecological range of wild barley differs in water availability, temperature, soil type, altitude, and vegetation generating a high potential of adaptive diversity to abiotic stresses. Genetic diversity and physiology in *H. spontaneum* from Israel and Jordan have been studied previously at *micro-* and *macrogeographic* scales. At the *macrogeographical* scales, adaptive genetic diversity was detected by protein (Nevo et al. 1997) and DNA markers (RAPD, SSR, and AFLP) (Pakniyat et al. 1997; Owuor et al. 1999; Turpeinen et al. 2001, 2003; Baek et al. 2003). At the *microgeographical* scales, local adaptive genetic diversity was found in several microsites in Israel (Ivandic et al. 2000, 2003; Gupta et al. 2003; Huang et al. 2002; Owuor et al. 1999). These adaptive genetic diversities indicate the potential of wild barley as a source for drought resistance alleles for breeding purposes. A wide range of well characterized genetic stocks across the Near Fertile Crescent is available at the Institute of Evolution, University of Haifa.

In the past decades, the relationships between genetic variation in morpho-physiological traits and adaptation of crop plants to drought conditions were extensively investigated (Blum 1988; Richards 1988; Ludlow and Muchow 1990; McDonald and Davies 1996; Passioura 1996). Important mechanisms of drought resistance mainly include the following: (1) drought escape via a short life cycle, photoperiod sensitivity, and developmental plasticity; (2) drought avoidance via enhanced water uptake and reduced water loss; (3) drought tolerance via osmotic adjustment (OA) and antioxidant capacity; and (4) drought recovery via desiccation tolerance (Nguyen et al. 1997; Zhang et al. 1999). More recently, the effects of drought at the cellular and molecular levels were studied (Bray 1997; Bajaj et al. 1999; Zhang et al. 2000; Bohnert and Bressan 2001; Knight and Knight 2001). A catalogue of traits for drought resistant measurements is proposed (Acevedo 1987; Turner 1997). However, it is difficult to identify traits that provide a consistent advantage of yield across variable water-limited environments (Teulat et al. 2003). In some cases the drought resistant traits were not related to yield, e.g., osmotic adjustment did not

show a positive effect on yield in wheat, barley, and maize (Serraj and Sinclair 2002). In many other studies traits having a positive relation with yield have been demonstrated. They include osmotic adjustment (Blum 1989; Morgan 1995; González et al. 2008), water-use efficiency measured through carbon isotope discrimination (Farquhar and Richards 1984), and relative water content (Singh and Patel 1996; Merah 2001). Although most of these traits are not useful for routine screening purposes because they are too time consuming, once DNA markers closely linked to the quantitative trait loci (QTLs) involved in the expression of a trait are identified, they can be used efficiently in a marker assisted selection (MAS).

QTL analysis seeks associations between quantitative traits and marker alleles segregating in the population. The basic theory for marker mapping has been available since the 1920s (Mather 1938), but had to be extended to handle hundreds of molecular markers simultaneously. Interval mapping (Lander and Botstein 1989) locates the likely site of the QTL. This has been the most widely used approach, particularly for those working with populations derived from inbred parents, because of the relative simplicity of the analysis, e.g., by using software MAPMAKER/QTL (Lincoln et al. 1992). In this study we employed a powerful user-friendly QTL mapping software package, MultiQTL (<http://www.multiqtl.com/>).

QTLs for drought resistant traits have been identified in many studies: for pre- and postflowering resistance to drought in sorghum (Tuinstra et al. 1996, 1997; Crasta et al. 1999); for anthesis-silking interval and yield components under drought in maize (Ribaut et al. 1996, 1997); for OA in rice (Lilley et al. 1996), barley (Teulat et al. 1998), and wheat (Morgan and Tan 1996); for relative water content in barley (Teulat et al. 2001, 2003); for abscisic acid (ABA) accumulation in wheat (Quarrie et al. 1994), maize (Lebreton et al. 1995; Tuberosa et al. 1998), and rice (Quarrie et al. 1997); for stomatal behavior in maize (Lebreton et al. 1995) and rice (Price et al. 1997); for root morphology and root penetration ability in rice (Champoux et al. 1995; Ray et al. 1996; Price and Thomas 1997; Yadav et al. 1997; Zheng et al. 2000). These QTLs were identified using the recombinant inbred lines (RILs), doubled-

haploid population (DH), F₂ population, and F₃ or F₄ families.

Large phenological difference was detected earlier between the two wild barley populations in Israel, Wadi Qilt (xeric) and Maalot (mesic), the former displaying a stronger earliness than the latter one (Nevo et al. 1984). Phenology has a powerful effect on plant growth and productivity under drought stress (Bidinger et al. 1987). The relative yield advantage of early genotypes under conditions of water stress has been concluded through the extended experience of plant breeders (Reitz 1974). More detailed studies (Jordan et al. 1983; Saeed and Francis 1983; Shakhtrah et al. 2001; González et al. 2008) have indeed shown that optimum maturity is related to environmental stress, where late-maturing genotypes were better adapted to wet conditions, while earlier-maturing genotypes were environment. Wadi Qilt wild barley population should employ both drought escape and drought resistance mechanisms. Therefore, our working hypothesis was that Wadi Qilt wild barley was better adapted to water stress conditions. To cope with the extreme xeric wild barley may be characterized by genetically determined seedling drought resistance. The objective of the present study is to reveal the molecular-genetic basis underlying drought resistance of xeric wild barley seedlings. QTL mapping of drought-resistance, morphological, and physiological traits of *Hordeum spontaneum* in Israel was conducted by using F₄ mapping populations derived from a cross of two wild barley lines, a xeric line Wadi Qilt (WQ23-38) and a mesic line Maalot (MA10-30). The advantage of such a cross compared to crosses between wild and cultivated barley include the ability to detect the alleles related to the natural adaptation to the contrasting environments, to find the favorable alleles even from mesic line for drought resistance, and to explore more genetic variation related to drought resistance.

Materials and methods

Plant materials and trait measurements

Drought resistant QTL mapping was conducted using 134 F₄ families derived from a cross of two wild barley lines, WQ23-38 and MA10-30, the former from Wadi Qilt, Judean Desert and the latter from Upper Galilee, Maalot, Israel, with 150 and 600 mm annual rainfall, respectively (Table 1). A “wilting and recovering” test was conducted with the F₄ families. Six seedlings each of the 134 F₄ families and 10 seedlings per parental line were grown and scored for twelve traits in a greenhouse.

Seeds were wet on filter paper in Petri dishes with distilled water followed by cold treatment at 4°C for 4 days and 25°C for 3 days. The consequent seedlings were scanned for measuring root length (RLE). Afterward the seedlings were transplanted to 0.55L plastic pot with sand, and irrigated once in 2 days with 100 ml per pot of nutrient solution: 1 mM Ca(NO₃)₂, 1 mM KNO₃, 0.5 mM KH₂PO₄, 0.5 mM MgSO₄, 25 μM Fe-EDTA, 3 μM MnCl₂, 4 μM ZnSO₄, 2 μM H₃BO₃, 1 μM CuSO₄. Seedlings were arranged next to each other randomly, and rearranged randomly every other day. After the establishment for 2 days in the pot, total leaf length (ll₁) of seedling was measured. When most of the seedlings showed the fourth leaves, water was withheld, at this time the total leaf length (ll₂) was measured again. Plant wilt was defined in this study as all leaves of the plant showing dehydration and turgor loss. The number of days from last irrigation to plant wilt was regarded as time to wilt (TW). After plant wilting total leaf length (ll₃) was measured and the last fully expanded leaf was sampled for osmolarity (OSM) and relative water content (RWC) measurements. Withered seedlings were watered with nutrient solution once a day for 3 days. Total leaf length (ll₄) was scored 3 days later. Then the seedlings were water withheld until all the

Table 1 Location of the two *Hordeum spontaneum* ecotypes and selected environmental data of sites of origin

Site of origin	Longitude (°E)	Latitude (°N)	Altitude (m)	Mean annual temp. (°C)	Mean annual rainfall (mm)	Mean humidity at 14:00 h (%)
Maalot	35.27	33.00	500	18	790	50
Wadi Qilt	35.38	31.83	50	23	144	35

shoots dehydrated. The dried shoots were removed and residues were watered. The shoot regrowth (REG) was measured 3 days later as height of new shoot growth. Trait measurements were summarized as following:

Drought-resistance traits

Time to wilt (TW, days), number of days after last irrigation when all leaves showed dehydration and turgor loss.

Leaf relative elongation rate (RER, %), $RER (\%) = (l_3 - l_2) \times 100 / (l_2 - l_1)$, where l_3 is total leaf length at wilting time, l_2 total leaf length at the last irrigation time, and l_1 total leaf length 2 days after transplanting of the seedling from a Petri dish to a pot.

Recovery rate (RCR, %), percent of new leaf length growth after water resuming, $RCR (\%) = (l_4 - l_3) \times 100 / l_3$, where l_4 total leaf length after 3 days growth after water resuming, l_3 total leaf length at wilting time.

Shoot regrowth (REG, cm), the height of new emerged shoot, was measured after 3 days growth after dried shoot removed and residue watered again.

Physiological traits

Osmolarity (OSM, mmol/kg), measured at the youngest fully expanded leaf (second or third from the top) using rehydration method (Chen et al. 2002) with Vapour Pressure Osmometer (5520, Wescor, USA). Basal part 1–1.5 cm length of the leaf was cut with a sharp scissor and immediately floated in distilled water in a Petri dish. After saturated at 4°C for 12 h, the leaf was dotted dry with tissue paper and put in a centrifuge tube following freezing in a –20°C refrigerator for 12 h. The leaf was thawed and centrifuged to get cell sap that was used to measure osmolarity.

Relative water content (RWC, %), $RWC (\%) = (FW - DW) \times 100 / (TW - DW)$, where FW is the leaf fresh weight, TW is the turgid weight, and DW is the dry weight. Leaf RWC was evaluated according to Barr and Weatherley (1962) at the same leaf that was used for osmolarity measurement.

Morphological traits

Leaf length: First leaf length (L1L, cm) and second leaf length (L2L, cm), measured at fully expanded first and second leaf numbering after coleoptile.

Root length (RLE, cm), measured at 3-day-old seedlings with Delta-T scan system (Delta-T Devices Ltd, UK).

Analysis of variance of all the measured traits and correlations analysis among the traits were conducted with STATISTICA for windows 5.1 D (StatSoft Inc., USA).

Construction of molecular framework map

A genetic map was constructed with pooled DNA samples extracted from leaves of 20 plants of each F₃ family whereas traits evaluation was conducted on the corresponding F₄ family. Methods of genotyping with SSR markers were employed as described by Peng et al. (1999, 2000) and Ramsay et al. (2000). AFLP analysis (Vos et al. 1995) was conducted using 21 Eco/Mse primer combinations. The method was modified by using fluorescent labeled primers, for visualization on an automated sequencer (Satish et al. 2001). AFLP polymorphic bands were designated according to the primer combination used and the size of the amplification products, i.e., band w3549-106: w indicating the band appearing in the WQ23-38, 3549 indicating the primer combination of E35 and M49, 106 meaning the size of 106 base pair. The genetic maps were constructed by means of Multi-Point program (<http://www.multiqt.com>) based on a new multilocus ordering algorithm (Mester et al. 2003). The map distances in the framework maps were computed using the Kosambi mapping function (Kosambi 1944).

QTL detection

To detect QTLs, the entire genome scanning was conducted for each of the quantitative traits using a general interval mapping approach (Lander and Botstein 1989; Korol et al. 1996). The analysis was based on mixture-model using ML-algorithms of the MultiQTL software (Korol et al. 2001). First, for each trait-chromosome combination, a single-QTL model was fitted and tested for significance. Then, the experimental-wise significance was evaluated for all

effects using the concept of false discovery rate (Benjamini and Hochberg 1995). The detected QTL effects were symbolized following the nomenclature described by McIntosh et al. (1998). In many cases, a clear coincidental mapping was found for different traits pointing to a pleiotropic effect of the corresponding QTL or tight linkage of several QTLs irresolvable with the sample size used in this study ($n = 134$). It should be noted here that, although the sample size $n = 134$ is rather modest, the F_2 phenotypes are in fact averages of F_4 families. Therefore, due to replicated progeny testing (Soller and Beckmann 1990), the resolution power of our design by far exceeds the one expected for $n = 134$.

Single-QTL model

In this case, the model can be presented as

$$x = \mu + v(g) + \varepsilon,$$

where $g = (QQ, qq, \text{ or } Qq)$ and $v(QQ) = D$, $v(qq) = -D$, $v(Qq) = h$, and ε is a random value with distribution $N(0, \sigma^2)$. Therefore, mean values for the QTL groups can be represented as $Ex_{QQ} = \mu + D$, $Ex_{qq} = \mu - D$, $Ex_{Qq} = \mu + h$, where D is the additive effect (hence $d = 2D = x_{QQ} - x_{qq}$ is the allele substitution effect at a QTL Q/q for trait X), and h is the dominance effect. On the basis of the relative magnitude of additive and dominance effects, a suitable simplified genetic model [“additive” ($h = 0$), “dominant” ($h = d/2$), or “recessive” ($h = -d/2$), or “general model” (h deviates significantly from 0, $d/2$ and $-d/2$)] was chosen for each of the putative QTLs. Standard permutation test (Churchill and Doerge 1994) was employed to obtain chromosome-wise statistical significance of the putative QTL revealed by fitting the single-QTL model. Based on 10,000 runs per chromosome, thresholds of the test statistics were obtained that served in further bootstrap analysis aimed at obtaining interval estimates of the main parameters (QTL position and effect, and the proportion of explained phenotypic variation).

Multiple-trait analysis

Multiple-trait complexes were established by trait grouping: multi-all meant that all the nine traits were included in the multiple-trait complex; multi-physi and multi-morph meant that the physiological and

morphological traits, respectively, were included in the corresponding complexes; multi-*rw*c indicated that the traits related with RWC were included in the complex, etc.

The main objectives of multiple-trait analysis in this study were: (a) to verify the QTL positions detected by single trait analysis and indicating pleiotropic effects, (b) to inspect the chromosome that displayed several coincidental effects that have not reached the single-trait significance threshold, (c) to summarize closely located single-trait QTL effects into a general multiple-trait QTL effect. A few different, though related, questions about the significance of the results can be recognized in the multiple-trait procedure (Korol et al. 2001): (1) Which traits significantly “contributed” to the criterion (multivariate LOD score)? (2) Which traits depend significantly on the chromosome under consideration? (3) What is the significance level of the detected QTL?

- (a) The first test aims to evaluate the significance of contributions of each of the traits to the test statistics, i.e., to multivariate LOD score. In the MultiQTL software, this test is conducted separately for each trait. For that, the individual values of the trait under consideration are reshuffled relative to the remaining data (the other trait values and marker scores). Clearly, some traits may prove to be insignificant because they contribute the same information as one (or a few) of the remainder traits. Thus, one can exclude insignificant traits from consideration by creation of a new trait set that does not include the insignificant trait(s).
- (b) The same procedure as in (a) can be used to test the significance of the QTL effect for each of the traits. Namely, we calculate the proportion of permuted cases where the estimated QTL effect d_i for the considered trait x_i fits the condition $\text{abs}(d_i) \geq \text{abs}(d^*_i)$, where d^*_i is the estimated effect on trait x_i obtained on initial (not reshuffled) data. The same criterion may be applied to the heterotic effect h_i or to the proportion of the explained phenotypic variance.
- (c) To evaluate the significance of the detected QTL for the trait complex, after removing the non-significant traits, the remaining complex of

traits was reshuffled relative to the marker scores. For each such permuted data set, the mapping procedure was applied resulting in a corresponding value of the test statistics (LOD score). This process was repeated 10,000 times and the significance of the H_0 hypothesis (no effect of the considered chromosome on the trait complex) was calculated as the proportion of permutation runs that resulted in LOD values equal to or exceeding the LOD* obtained on the non-permuted data.

Based on the permutation runs per chromosome, the thresholds of the test statistics were obtained for bootstrap analysis to calculate the QTL detection power, and the standard deviations of the QTL position and the proportion of explained multivariate phenotypic variation.

Multiple interval mapping

The ‘Multiple Interval Mapping’ algorithm implemented in MultiQTL software reduces the residual (non-controlled) variation by taking into account QTL effects from other chromosomes (Jansen and Stam 1994; Zeng 1994; Kao et al. 1999). It consists of sequential chromosome analysis for QTL presence while subtracting the QTL effects of other chromosomes. Since the positions and effects of these QTLs are unknown in advance, the algorithm is iterative. First, the most powerful QTL is found, and its effect

is subtracted. Then, the next most powerful QTL is searched and its effect is subtracted, and so on. Assuming no interaction, the QTLs effects are re-evaluated by fitting the QTLs from other chromosomes in the order of their power. This procedure is applied repeatedly until the difference between the parameters of each QTL on two consecutive iterations is less than a preselected value.

Results

Expression of the traits

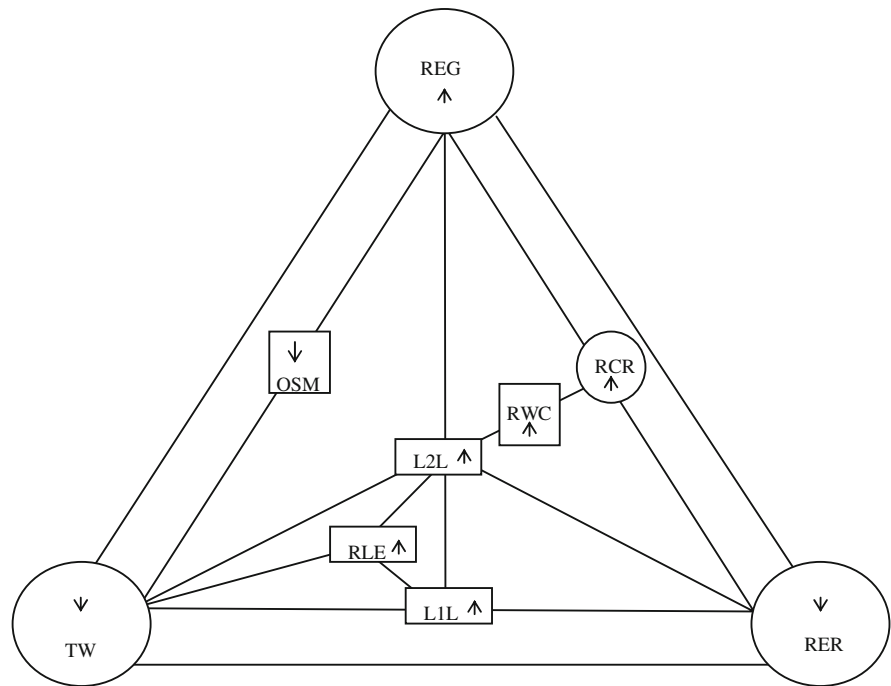
The traits scored in the present study are listed in Table 2. Four drought-resistance traits were scored: time to wilt (TW), leaf relative elongation rate (RER), recovery rate (RCR), and regrowth rate (REG). In addition, we scored two physiological traits: osmolarity (OSM) and relative water content (RWC); and three morphological traits: first leaf length (L1L), second leaf length (L2L), and root length (RLE). The three morphological traits exhibited significant difference between two parents, xeric *H. spontaneum* WQ23-38 and mesic *H. spontaneum* MA10-30, while four drought resistance traits and two physiological traits showed no significant difference between the two parents. Genotype WQ23-38 had longer first and second leaf length and root length than genotype MA10-30. Although the difference

Table 2 Phenotypic means, standard deviation (SD) and ranges of variation (minimum-maximum values) of the 134 F_4 families and the two parental genotypes for drought resistance traits

Trait	MA10-30			WQ23-38			F ₄ families				Significance of family effect	Significance of parental effect
	Mean	SD	Range	Mean	SD	Range	Mean	SD	CV (%)	Range		
RWC	45.2	5.5	36.5–58.8	48.2	5.5	39.5–57.2	44.7	6.8	15.2	25.2–71.6	**	ns
OSM	552	46	451–608	562	29	495–589	595	69	11.6	257–830	***	ns
L1L	12.5	0.8	10.9–14.0	14.1	2.2	9.7–17.0	12.8	1.8	14.1	6.6–18.7	***	***
L2L	16.6	1.5	13.2–18.9	19.3	3.4	12.5–22.4	18.0	2.5	13.9	10.1–25.7	***	***
RLE	6.6	0.4	6.2–7.1	7.8	0.3	7.3–8.0	6.5	1.4	21.5	1.0–10.5	***	*
TW	6.8	0.7	6–8	6.6	0.7	6–8	6.9	0.7	10.1	6–8	**	ns
RER	57.2	9.1	42.7–71.4	53.8	4.4	46.2–70.0	58.4	9.1	15.6	32.7–99.7	***	ns
RCR	8.2	1.2	6.0–10.2	8.8	1.1	7.4–10.3	7.7	2.2	28.6	1.2–15.2	*	ns
REG	2.8	1.3	0–6	3.0	0.7	1–6	2.2	1.7	77.3	0–12	*	ns

Notes: TW time to wilt, RER leaf relative elongation rate, RCR recovery rate, REG regrowth rate; physiological traits: OSM osmolarity, RWC relative water content; and morphological traits: L1L first leaf length, L2L second leaf length, RLE root length, The significances of F₄ family and parental effects were obtained from an analysis of variance

Fig. 1 Relationships among traits. Drought resistant traits (*circle*): time to wilt (*TW*), leaf relative elongation rate (*RER*), recover rate (*RCR*), regrowth rate (*REG*); physiological traits (*square*): osmolarity (*OSM*), relative water content (*RWC*); morphological traits (*rectangle*): first leaf length (*L1L*), second leaf length (*L2L*), and root length (*RLE*). Trait measurements are described in the section “Materials and Methods”. Lines connecting two traits indicate significant correlation ($P < 0.05$). Arrows indicate the association among the traits



between parental lines was not significant in six drought resistance and physiological traits, obvious segregation was observed for all the nine measured traits in the mapping populations (Table 2). The coefficient of variation (CV) ranged from 10.1 to 77.3%. The distributions of all the measured traits showed strong transgressive segregation, suggesting that the mesic genotype also contains positive alleles for drought-resistance traits.

Trait relationships based on F_4 families scores (Fig. 1) display some tradeoff among drought resistant traits: increased REG and RCR, while TW and RER are decreased. No direct relationship was revealed between two physiological traits, but indirectly, OSM decreased while RWC increased. Significant positive correlations were found among morphological traits. Drought resistance traits were related to physiological traits and morphological traits; REG was negatively related to OSM and positively to L2L. RCR was related to RWC, RER to L1L and L2L. TW was related positively to OSM and negatively to three morphological traits.

Construction of genetic linkage map

SSR and AFLP markers and one gene, *Hsdr4* (Suprunova et al. 2007), were used to construct the

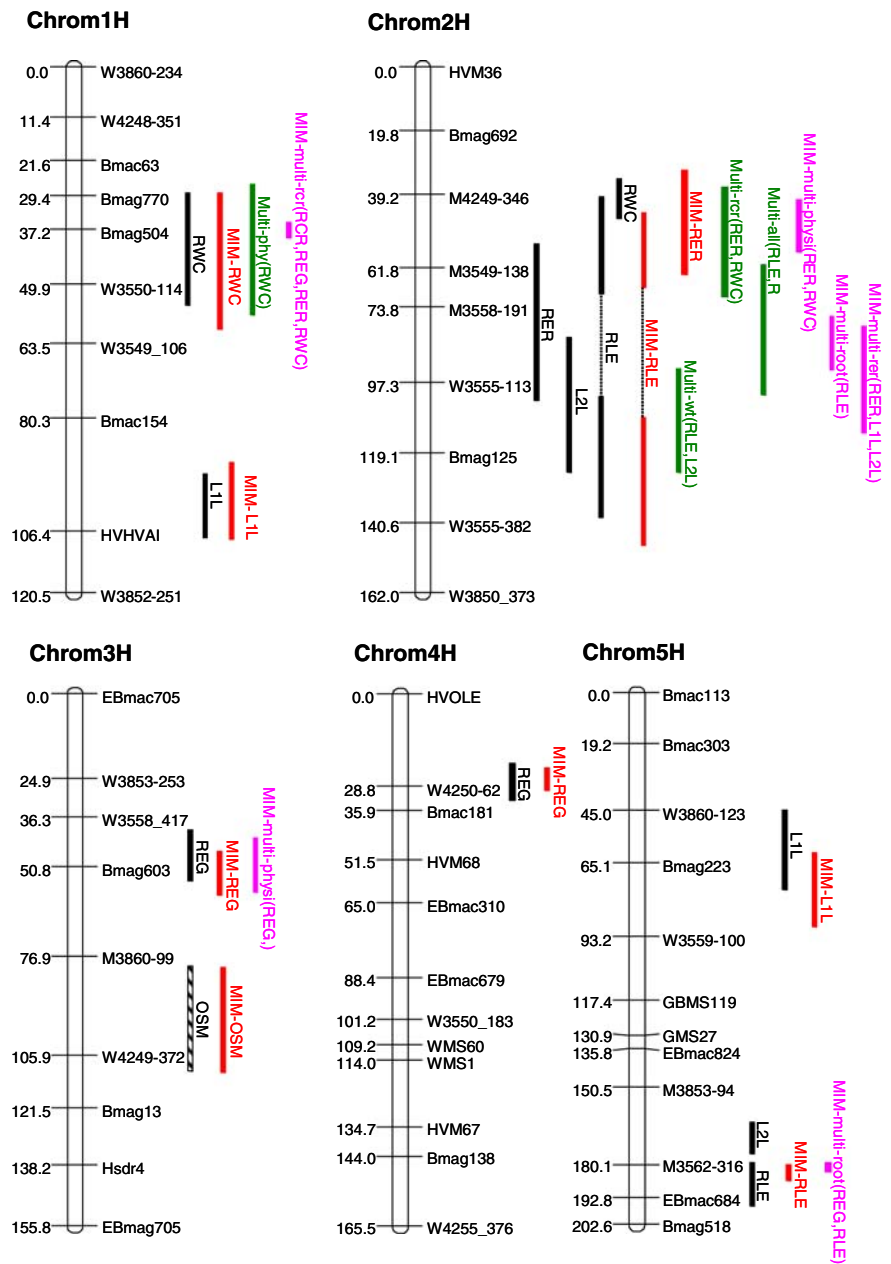
genetic linkage map of *H. spontaneum* based on F_4 mapping population derived from a cross between WQ23-38 (xeric genotype) and MA10-30 (mesic genotype). Among 187 SSR markers tested, 50 were polymorphic between the two parents and 37 were used to construct the genetic linkage map. AFLP analysis revealed 193 polymorphic bands, and 35 of those polymorphic bands were mapped on the genetic linkage map. The total length of the map was 1,085 cM with an average interval of 16.4 cM (Fig. 2).

Detected QTLs

Single-trait analysis with single-QTL-per-chromosome model was employed to detect QTL. When the false discovery rate was controlled at 10%, sixteen putative QTLs were detected (Fig. 2; Table 3). These QTL effects were confirmed by Multi-trait, MIM-multi-trait, and MIM-single-trait analysis (Fig. 2).

TW: One QTL effect was detected on chromosome 7H (Table 3), $P = 0.0027$, explaining 19.5% of the phenotypic variation. Beneficial allele from xeric WQ23-38 increased the time to wilt of the plant under drought stress.

Fig. 2 Molecular genetic map and QTL locations of *Hordeum spontaneum* based on F₄ families from a cross of Wadi Qilt (xeric ecotype) × Maalot (mesic ecotype). The map distances are expressed in cM (Kosambi function) on the left of the chromosome skeleton, the markers and QTL on the right. QTL is shown by $L \pm SD/2$, whereas L is the position and SD the standard deviation, and indicated by a bar (a SD in length); a shaded bar indicated a non-significant QTL. The dashed lines that connected two bars indicated two linked QTLs. The QTLs identified by single-trait analysis are indicated by the *trait name* whereas those identified by multi-trait analyses are indicated by the *model name* following by the traits (the traits that were significantly affected by the QTL were *bolded*). The traits include time to wilt (*TW*), leaf relative elongation rate (*REG*), recovery rate (*RCR*), regrowth rate (*REG*); physiological traits: osmolarity (*OSM*), relative water content (*RWC*); morphological traits: first leaf length (*L1L*), second leaf length (*L2L*), and root length (*RLE*)



REG: One QTL effect was detected on chromosome 2H (Table 3), $P = 0.0011$, explaining 52.6% of the phenotypic variation. It gained beneficial allele from mesic MA10-30.

RCR: One QTL effect was detected on chromosome 7H, explaining 57.5% of phenotypic variation. The allele from the xeric parent contributed the positive effect on new leaf length growth after rehydration of drought-stressed plants (recovery rate).

REG: Three QTL effects were detected on chromosome 3H, 4H, and 6H. They explained from 19.0, 34.4, and 16.6% of the phenotypic variation. The allele on chromosome 4H from xeric parent contributed the positive effects on 3-day regrowth after dried shoot removed and residue rewatered.

RWC: Three QTL effects were detected on chromosome 1, 2, and 6H. The allele on chromosome 2H from xeric parent contributed the positive

Fig. 2 continued

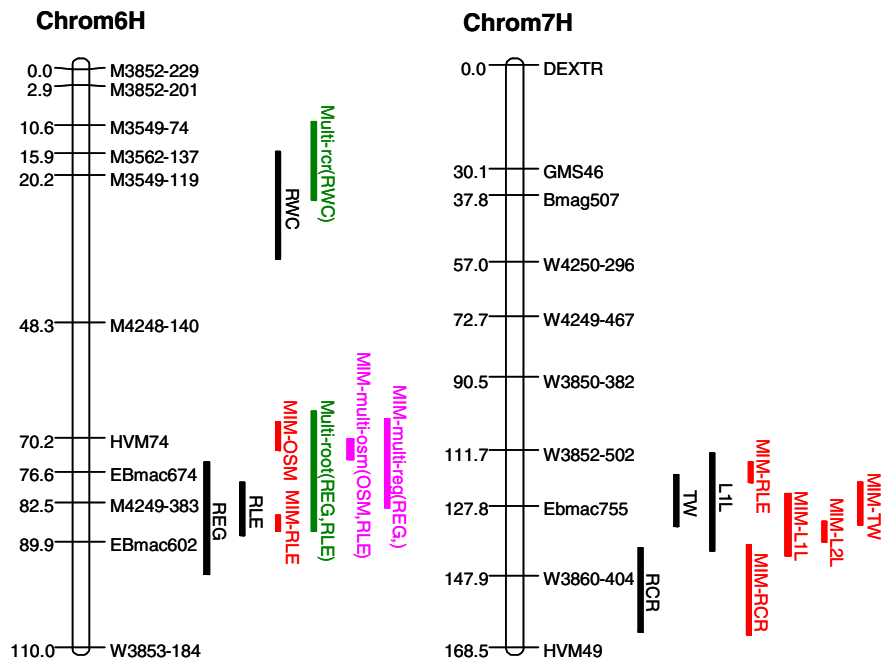


Table 3 QTLs detected by single-trait analysis with single-QTL-single-chromosome model and two-QTL-single-chromosome model

Chrom.	Trait	LOD	<i>P</i> -value	Power	<i>L</i> (cM)	PEV (%)	Submodel	<i>d</i>	<i>h</i>
Chrom 1	L1L	4.18	0.0024	0.73	100.8 ± 14.8	0	het	0	1.83 ± 0.72
Chrom 1	RWC	2.48	0.0066	0.65	41.9 ± 25.8	16.4 ± 4.7	add	-3.55 ± 0.60	0
Chrom 2	RER	3.73	0.0011	0.96	78.7 ± 48.9	52.6 ± 16.9	gen	-6.27 ± 1.92	-8.97 ± 4.21
Chrom 2	L2L	3.02	0.0025	0.88	104.1 ± 41.8	32.9 ± 12.5	dom	2.25 ± 0.53	1.12 ± 0.26
Chrom 2	RLE	7.80	0.005*	0.99	55.0 ± 29.8	14.3 ± 6.5	het	0	-1.60 ± 0.42
					120.3 ± 37.4		dom	1.34 ± 0.29	0.67 ± 0.15
Chrom 2	RWC	3.28	0.0006	0.83	40.5 ± 11.9	25.1 ± 7.3	rec	3.66 ± 0.71	-1.83 ± 0.36
Chrom 3	REG	3.47	0.0005	0.90	47.4 ± 15.0	19.0 ± 5.8	add	-0.90 ± 0.22	0
Chrom 4	REG	4.30	0.0005	0.73	27.2 ± 11.6	34.3 ± 12.0	rec	1.08 ± 0.34	-0.54 ± 0.17
Chrom 5	L2L	6.54	0.0001	0.98	169.9 ± 11.8	52.2 ± 12.4	dom	3.07 ± 0.56	1.53 ± 0.28
Chrom 5	RLE	3.85	0.0005	0.85	187.4 ± 16.9	30.4 ± 11.0	dom	1.42 ± 0.33	0.71 ± 0.16
Chrom 5	L1L	3.02	0.0028	0.78	59.9 ± 30.6	17.4 ± 5.8	add	1.38 ± 0.28	0
Chrom 6	REG	2.07	0.0150	0.55	85.6 ± 21.1	16.6 ± 4.3	rec	-0.71 ± 0.11	0.35 ± 0.06
Chrom 6	RLE	2.12	0.0173	0.53	83.8 ± 10.2	15.8 ± 4.6	add	1.22 ± 0.23	0
Chrom 6	RWC	2.04	0.0147	0.58	25.9 ± 20.2	22.6 ± 5.4	dom	-3.45 ± 0.52	-1.72 ± 0.26
Chrom 7	RCR	3.17	0.0169	0.55	152.0 ± 24.2	57.5 ± 10.5	gen	0.36 ± 0.40	1.84 ± 0.62
Chrom 7	WT	3.10	0.0027	0.69	126.1 ± 14.5	19.5 ± 5.7	dom	0.34 ± 0.10	0.17 ± 0.05
Chrom 7	L1L	3.01	0.0022	0.71	126.6 ± 28.6	16.8 ± 5.4	add	-1.35 ± 0.35	0

Notes: *L* QTL position, *d* substitution effect, *h* heterotic effect. Significant threshold was 0.05, FDR at 0.10, power estimation at *P* = 0.01. *Chrom.* is chromosome, *PEV* is percentage explanation of trait variation. *Rec* recessive, *dom* dominant, *add* additive, *het* heterotic, *gen* general. QTL effect was showed by substitution effect with the sub model of rec, dom, add and gen and by heterotic effect with heterotic sub model

effect on relative water content of drought-stressed leaves.

L1L: Three QTL effects were detected on chromosome 1, 5, and 7H. The WQ23-38 allele on chromosome 5H could increase the trait value, whereas the MA10-30 allele on chromosome 7H increased L1L.

L2L: Two QTL effects were detected on chromosome 2 and 5H, explaining 32.9 and 52.2% of phenotypic variation, respectively. The WQ23-38 alleles at both QTLs contributed the positive effects on the second leaf growth.

RLE: Four QTL effects were detected on chromosome 2H, 5H, and 6H. WQ23-38 alleles at the four QTLs increased RLE trait value.

Discussion

Drought resistant traits and their relationship

In the botanical domain the interpretation of drought resistance requires an understanding of the contribution of the traits to ontogenetic survival. However, in the agronomic domain this interpretation requires the traits in terms of plant production and its sustainability under drought stress (Blum 1996). It is important to study the physiological or the genetic factors controlling the traits preferred in both domains and apply it in plant breeding programs. Leaf relative elongation rate (RER), time to wilt (TW), recovery rate (RCR), and regrowth (REG) were considered as drought-resistance traits because an ideal drought resistant plant, under drought stress, should have high relative growth, postponed wilting, high recovery rate after rehydration, and the ability for regrowth after severe drought stress. Late wilted seedlings had a relative long growth time after water withholding, thus they had a high relative leaf elongation rate. Withstanding drought stress for a longer time, late wilted seedlings suffer more from soil drying and leaf transpiration, therefore, they had lower recovery rate and regrowth. These tradeoffs were demonstrated in Fig. 1. In nature, wild barley seedlings respond to drought with different strategies under different environments. To withstand severe drought, it adopts the “survival strategy” by wilting early to reduce evapotranspiration to protect plant growth point underground, keeping the high ability to recover

and regrowth. To withstand moderate drought, it employs the *growth-sustaining strategy* by maintaining growth under drought stress. For agricultural exploitation, the barley breeder should target survival traits, REG and RCR, in arid and/or semiarid area, and target growth-sustaining traits, RER and TW, in semi-humid drought prone area.

We found that osmolarity (OSM) was related to time to wilt (TW). A high OSM means a low osmotic potential that results in postponing plant wilting. A trait is important for drought resistance when a QTL effect for this trait benefits a higher RWC under stress, maintenance of RWC and an OA capacity, being in favor of turgor maintenance and contributing to yield stability under drought conditions in cereals (Clarke and McCaig 1982; Morgan 1983; Schonfeld et al. 1988; Matin et al. 1989; González et al. 2008). Although RWC and TW did not show significant direct correlation, as described in Fig. 1, they demonstrated indirect negative relationship via L2L, i.e., early wilting leaves had a higher RWC than late wilting leaves.

The xeric WQ 23-38 was characterized by narrow (data not shown) and long L1L, L2L at the seedling stage, and thin (data not shown) and long root at post germination stage. These morphological traits positively related with REG, RWC, negatively with TW and RER, indicating that the WQ23-38 line had the tendency to wilt early, to have low RER, but high RWC and REG, although these traits were not significantly different between WQ23-38 and MA10-30.

Short first and second leaf length and short root length indicate small plant size. Small plants consume less water, sustain longer after water withholding, and get more time to adapt drought stress, to adjust osmotic potential and other process. Small plant size, high osmolarity, and low water content at the leaf wilting point are favorite traits for late wilting and high relative leaf elongation rate. Large plant size, low osmolarity, and high water content at leaf wilting point are favorite traits for recovery rate and regrowth.

Drought resistant traits are not consistent with each other; it is important to take all traits into account and to target a certain environment when one evaluates a genotype for drought resistance. Blum (1996) pointed out that the ideal genotype for drought resistance depends on our ability to predict

environmental effects on plants and plant responses to stress, in the context of a given agro-ecological system.

QTL identification with multiple models

Multi-trait analysis, MIM-single-trait analysis, and MIM-multi-trait analysis could not only confirm the QTLs detected by Single-trait analysis, but also identify new QTLs that Single-trait analysis failed to identify (Table 3; Fig. 2). This is also indicated by Zhang et al. (2001). Multi-trait analysis (Korol et al. 2001) is (a) to verify the QTL positions detected by single trait analysis and indicating pleiotropic effects, e.g., QTL multi-root verifying QTL REG and RLE in Chrom6H; (b) to summarize closely located single-trait QTL effects into a general multiple-trait QTL effect, for example, in Chrom2H, QTL Multi-all summarizing closely located QTL RLE, L2L, and RER. The ‘Multiple Interval Mapping (MIM)’ reduces the background (non controlled) variation by taking into account QTL effects from other chromosomes (Jansen and Stam 1994; Zeng 1994; Kao et al. 1999). Therefore, MIM QTLs are more accurate than the QTLs identified by other method. Most of the MIM-single-trait QTLs had smaller standard deviation (shorter bar) for loci position than their related Single-trait QTLs (Fig. 2).

The genetic bases of drought resistant traits and their relationship

Both QTL effects detected for L2L showed that WQ23-38 had positive substitution effects (Table 3), indicating that WQ23-38 alleles increased L2L trait value. Similarly, WQ23-38 alleles increased RLE trait value. These results corroborated the fact that L2L and RLE scores of WQ23-38 were significantly higher than those of MA10-30 (Table 2) and that L2L and RLE were positively correlated with each other (Fig. 1).

The substitution effects of WQ23-38 alleles at QTL for time to wilt and recovery rate were positive and that for relative leaf elongation rate was negative. Therefore, WQ23-38 alleles increased L2L, RLE, and RCR and decreased RER, in other word, MA10-30 alleles decreased L2L, RLE, and RCR and increased RER, which indicated that L2L, RLE, and RCR were positively correlated with each other and negatively

correlated with RER. This was in agreement with the correlation among these traits showed in Fig. 1. However, the substitution effects of WQ23-38 allele at QTL for OSM and TW were positive, so was that for L2L (Table 3), indicating that OSM and TW were positively correlated with L2L. This was not in agreement with the correlation among these three traits (Fig. 1). There might be two reasons for this disagreement. Firstly, OSM and TW showed the lowest coefficient of variation, 11.6 and 10.1%, respectively (Table 2), suggesting that OSM and TW trait segregation might be not enough to get strong trait correlation and QTL effect. There might be some weak QTL effects not identified by single-trait analysis. Secondly, there might be not enough QTL effect for OSM and TW to be identified (Fig. 2). It was difficult to determine whether WQ23-38 alleles at QTL for RWC and REG exert positive or negative substitution effects in general due to similar in both positive and negative substitution effects.

Collocations of the present and known QTL effects for drought resistant traits

Three QTL effects for RWC were detected on chromosome 1H, 2H, and 6H. Previously ten genomic regions for RWC were identified in barley chromosomes 1, 2, 4, 5, 6, and 7H (Teulat et al. 2003). In rice, eleven QTLs on nine genomic regions for RWC measured in two different environments (Courtois et al. 2000) and eight QTLs for RWC scored in three different environments (Price et al. 2002) were identified. The QTL effect for RWC on chromosome 1H was collocated with an effect for relative water content in drought-stressed plants (RWC_s) (Teulat et al. 2001) and a QTL effect for plant drought tolerance (Cattivelli et al. 2002). The QTL effect for RWC on chromosome 6H was coincident with a QTL effect for RWC in field-grown barley (Teulat et al. 2003) and a QTL effect for grain carbon isotope discrimination in field-grown barley (Teulat et al. 2002).

QTL effects for REG on chromosome 3H might match the QTL effects for biological yield, grain yield, and 1,000-kernel weight under drought stress (Baum et al. 2003). Our QTL for REG on chromosome 4H coincides with a QTL for RWC under Mediterranean field conditions (Teulat et al. 2003). The genomic region controlling REG on chromosome

6H was previously identified as controlling leaf osmotic potential and osmotic potential at full turgor, with OA as well as RWC measured under water-deficit conditions and RWC scored under Mediterranean field conditions (Teulat et al. 1998, 2001, 2003). This region was also related to a drought tolerant QTL and a cold tolerant QTL (Cattivelli et al. 2002). The homoeologous region of rice chromosome 2 was also identified as involved in OA (Zhang et al. 2001). These coincidences indicate that the region near EBmac602 on chromosome 6H is a conserved genomic region that controls plant reactions to drought stress.

The QTL effects for RER, L2L, and RLE on chromosome 2H near AFLP marker W3555-113 might match a QTL effect for RWC under Mediterranean field conditions (Teulat et al. 2003) and a QTL effect for salt tolerance (Cattivelli et al. 2002). This region on chromosome 2H might be important for determination of barley seedling morphology since it controlled both morphology of young shoots and roots. This region was also related to abiotic stress tolerance. Therefore, the seedling morphology might, to some extent, predict abiotic stress tolerance.

In conclusion, xeric and mesic genotypes adopt survival and growth-sustaining strategies, respectively, to cope with drought stress. A high recovery rate and regrowth rate after rehydration of drought stressed seedlings characterized the drought resistance of xeric genotypes. Wild barley drought resistance may be attributed to dehydrin expression, osmotic adjustment, and relative water content preservation. Although drought adaptation in wild barley involves the induction of a large number of genes, comparative genetics have revealed a genomic region near Ebmac602 on chromosome 6H for drought tolerance that is consistent across multiple genetic backgrounds and environments. One might infer that the genes residing in this region may encode highly conserved regulatory factors that have global effects on gene expression related to abiotic stress tolerance. The conserved regions on chromosome 6H controlling abiotic resistance is worthy of further study.

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