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## QTL for relative water content in field-grown barley and their stability across Mediterranean environments

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**Abstract** A quantitative genetics approach was developed to identify the genomic regions that control relative water content (RWC) in field-grown barley. The trait was previously demonstrated to be a relevant screening tool of drought-tolerance in cereals, as well as a good indicator of plant water-status. The trait was measured at the heading stage on flag leaves recorded from 167 recombinant inbred lines grown in several Mediterranean sites (Montpellier, France; Meknès, Morocco; Le Kef, Tunisia). The results obtained confirmed that several genomic regions are implicated in the total phenotypic variation of RWC. A total of nine chromosomal regions were identified. One region situated on the long arm of chromosome 6H contains the most-consistent QTL obtained in the present study. This region was previously identified as controlling RWC, as well as leaf osmotic potential under water stress and osmotic adjustment, from an experiment conducted in growth-chamber conditions with the same genetic background. The confirmation of the role of this region in the genetic control of water and turgor status underlined its interest for breeding purposes in the Mediterranean area.

In addition, the presence of several dehydrin loci in the same chromosomal area reinforce its interest for genomics analyses to confirm, or not to confirm, the implication of these genes in the variation of RWC.

### Introduction

The most difficult task for cereal breeders in Mediterranean countries is to develop varieties able to tolerate drought stress fluctuating across years and environments, by improving yield-stability. Genotype by environment effects acting on yield decrease breeding efficiency and to overcome this problem, huge trials have to be conducted across years and environments to identify the most-suitable genotypes. This type of strategy is time-consuming and have conducted several authors to study criteria that have a positive influence on yield and yield-stability in the Mediterranean basin. A catalogue of traits is proposed (Acevedo 1987; Turner 1997). However it is difficult to identify traits that provide a consistent advantage on yield across variable water-limited environments.

The traits where a positive relation with yield has been demonstrated in cereals are osmotic adjustment (Blum 1989), water-use efficiency measured through carbon isotope discrimination (Farquhar and Richards 1984) and relative water content (Schonfeld et al. 1988; Tahara et al. 1990; Peltonen-Sainio and Makela 1995; Singh and Patel 1996; Merah 2001). Traits allowing the evaluation of plant water-status, and particularly relative water content (RWC), could give an idea of the level of the water deficit in the plant at a specific time-point. As RWC is related to cell volume, when it is measured on the flag leaf, it may closely reflect the balance between water supply to the leaf and transpiration rate (Sinclair and Ludlow 1985). The potential value of RWC for breeding under drought-stress conditions has been demonstrated by Schonfeld et al. (1988) in winter bread wheat. These authors also showed that RWC is inherited quantitatively and controlled by genes with additive effects. A wide variation

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was observed by Tahara et al. (1990) and Martin et al. (1997) in bread wheat, by Merah (2001) in durum wheat, by Peltonen-Sainio and Makela (1995) in oat and by Martin et al. (1989), Arnau and Monneveux (1995) and Teulat et al. (1997a) in barley. Finally, a positive correlation between grain yield and RWC have been observed in durum wheat (Merah 2001), in bread wheat (Schonfeld et al. 1988; Tahara et al. 1990; Singh and Patel 1996) and in oat (Peltonen-Sainio and Makela 1995).

Genetic analysis of complex characters such as drought-tolerance allows the identification of quantitative trait loci (QTLs) which through marker-assisted-breeding may ultimately improve selection efficiency for yield. The yield-selection criterion, such as RWC, is one of the traits linked to drought-tolerance which could be efficiently tracked by molecular markers. The use of molecular markers as selection criteria would also represent an important surrogate for physiological trait measurements (Price and Courtois 1999). Curiously, scarce-works reported molecular markers associated with traits related to plant water-status in cereals. Several QTLs were however reported for the Mediterranean barley segregating-population issued from the cross between Tadmor and Er/Apm (Teulat et al. 1997b, 2001a). Moreover, the latter work was conducted in controlled conditions and at a young stage of growth. Lafitte and Courtois (1999) have underlined the need to verify QTL results obtained from "artificial" conditions with the field situation, which are the real agronomic conditions.

The overall aim of the work presented in the paper, was to identify QTLs for relative water content, a trait evaluating plant water-status, measured at the heading stage in Mediterranean field conditions and their stability across several environments in the recombinant inbred line population, derived from the cross between two Mediterranean-barley parents. In a second step, the QTLs identified were compared to the ones obtained with the controlled-condition experiment conducted on the same genetic background (Teulat et al. 2001a).

## Materials and methods

### Plant material, site and Crop Management

A progeny of 167 two-row barley (*Hordeum vulgare* L.) recombinant inbred lines (RILs), and the two parents Tadmor (selected by ICARDA from Arabi Aswad, a Syrian landrace) and Er/Apm (selected line, released in Tunisia) were grown in three Mediterranean sites (Montpellier, France; Meknès, Morocco and Le Kef, Tunisia). The trial in Montpellier was conducted under two water treatments in 1999 (F99rain: rainfed conditions, and F99ir: with an irrigation supply) in the experimental field at ENSAM campus in a random complete block design with two blocks per water treatment. For the two other sites, the same design was used and the plants were grown under rainfed conditions, in Meknès at the experimental field of ENA successively in 2000 and 2001 (M00 and M01 respectively) and in Le Kef at the experimental station of INRAT in 2001 (T01). Details of the geographic situation and the edaphic characteristics of each site are described in Table 1. The plants were sown on November 20th 1998 for M99rain and M99ir, on

November 28th 1999 for M00, on November 12th 2000 for M01 and on November 11th 2000 for T01. In M99, two rows per line were grown for each block, 101 kg/ha of PK was supplied before sowing, and then 124 kg/ha of Nitrogen (Ammonitrate) was added twice. In M00 and M01, 80 U/ha of Triple Super Phosphate (45%) and potassium sulfate (50%) was added before sowing, as well as 1/3 of 100 U/ha of Ammonitrate (33.5%). The other 2/3 of Ammonitrate were added during tillering. In T01, 100 U of Triple Super Phosphate (45%) and 33.3 U of Ammonitrate (33.5%) was added before sowing. For M00 and T01, six rows 3.5-m long and 25-cm spaced were sown, leading to plots of 5.25 m<sup>2</sup> with a seeding rate of 240 seeds per m<sup>2</sup>. Then for M01, the plots were reduced to 4 m<sup>2</sup> with a seeding rate of 300 seeds per m<sup>2</sup> with, for each plot, four rows 3-m long and 25-cm spaced.

### Climatic conditions

F99rain, F99ir, M00, M01 and T01 were considered as five different environments (E). These environments differed in total cumulative rainfall (and water supply for F99ir) (Table 1) as well as rainfall distribution. In F99rain, plants received 360 mm. For this environment, only a third of the cumulative rainfall fell during the 3 first months. The main drought period was in February 1999 with 0 mm. The plants of the irrigated blocks (F99ir) received 98 mm of additional water in six applications so that the two environments F99rain and F99ir differed in plant water-availability (see Teulat et al. 2001b for details). M00 was characterised by a hard drought period with 3 months without any rainfall (January to March) and high temperatures. An irrigation supply was necessary to maintain the plants alive: 80 mm were added during two periods (40 mm between February 17th and 27th, and 40 mm between March 12 and 19th). For M01, after December and January cumulating 165.6 mm (62% of the total rainfall), the trial was characterised by a long drought period from February to May where the plants were harvested. Finally, T01 was also characterised by a long drought period from January to May. For all environments, high rates of evapo-transpiration (ETP) were noticed particularly at the end of the growth cycle. This leads to low ratios of rainfall (R) on ETP (R/ETP) characterising drought stress.

### Measurements

Relative water content (RWC) was measured at heading for all environments. In F99rain and F99ir, four different flag leaves per line were recorded in the two rainfed blocks and in one block in the environment with an irrigation supply. For the other environments, five flag leaves per plot (i.e. per line) in each block were sampled at random and weighted together. In all environments the same procedure was then followed. The fresh weight was immediately recorded after leaf excision (FW). The leaves were left in distilled water for 24 h at 4°C in darkness and the turgid weight was recorded (TW). The dry weight (DW) was then measured after 48 h at 80°C. The RWC was calculated according to Barrs and Watherley (1968) as  $RWC (\%) = [(FW - DW) / (TW - DW)] \times 100$ .

### Statistical analyses

Data of RWC were subjected to an analysis of variance using the GLM (General Linear Model) procedure of SAS (SAS institute 1987). The genotype (RIL), and when possible, the block effects were evaluated. For the four rainfed the environments, adjusted entry means were generated by fixing the block within the environment effect and used for QTL analyses.

### QTL detection

The genetic map used for QTL analyses was described in Teulat et al. (2002). It comprises 133 molecular markers mostly RFLPs and

**Table 1** Environment characteristics: situation, soil composition, rainfalls (R), temperature (T), evapotranspiration (ETP) and R/ETP in all environments for the whole growth cycle and for the period of ten days before relative water-content measurements

Environment	F99rain (Montpellier)	F99ir (Montpellier)	M00 (Meknès)	M01 (Meknès)	T01 (Le Kef)
Situation	43°36'N 3°52'E 45 m	43°36'N 3°52'E 45 m	33°53'N 5°37'O 600 m	33°53'N 5°37'O 600 m	36°14'N 8°27'E 518 m
Edaphic characteristics					
Clay	18.7	18.7	50.8	50.8	52.5
Lime	32.0	32.0	13.0	13.0	12.5
Sand	49.3	49.3	14.0	14.0	35.0
CaCO <sub>3</sub>	–	–	5.2	5.2	26.8
pH (H <sub>2</sub> O)	7.8	7.8	8.0	8.0	8.0
Organic matter	2.1	2.1	2.5	2.5	–
Climatic characteristics for all the growth cycle (from sowing to harvest)					
R (mm)	360.5	458.0	254.6	267.4	282.8
T (°C)	12.035	12.035	14.394	12.9	14.47
ETP (mm)	335.4	335.4	521.78	434.24	488.2
R/ETP	1.075	1.366	0.487	0.616	0.579
Climatic characteristics for the period of 10 days before measurements					
R (mm)	0.5	38	0	4.3	34.7
T (°CD)	159.4	127.5	175	144	144.5
ETP (mm)	28.2	21.6	32.83	26.9	39.28
R/ETP	0.018	1.76	0	0.16	0.88

SSRs, scattered along the seven barley chromosomes. QTL analyses were performed on RIL-means adjusted for the block effects within each environments (except for F99ir). The marker-trait associations were performed with simple interval mapping (SIM) and with composite interval mapping (CIM) using the PLABQTL software package (Utz and Melchinger 1995). During SIM, 1,000 permutations were performed to identify the LOD (logarithm of the odds ratio) threshold to use to consider experiment-wise risks of 5% and 10%, corresponding respectively to an individual type-I risk of 0.27% and 0.55%. The global 10% risk was considered as a compromise between the type-I risk and the power of detection, and for the identification of QTLs for water-status parameters measured in Mediterranean field conditions. Considering these risks, the significant LOD thresholds varied from 2.80 to 2.91 for a global error of 5%, and from 2.46 to 2.57 for a global error of 10%. These thresholds were used for both SIM and CIM analyses. For CIM, a maximum of six cofactors was considered. Adjusted means by environmental effects T, rainfalls, ETP and R/ETP were also considered for QTL detection. In addition, SIM and simplified composite interval-mapping (sCIM) analyses were performed using the software package MQTL, adapted for the evaluation of progeny in multiple environments to identify QTLs that either do or do not exhibit QTL×E interaction (Tinker and Mather 1995a, b). For sCIM, 40 background markers well scattered along the chromosomes, and chosen near QTLs detected by PLABQTL for the trait studied, were used as cofactors to control the effect of the genetic background. A test statistic (TS) value (Haley and Knott 1992) was produced for both the QTL main effect across E, and QTL×E interaction at each marker locus for both SIM and sCIM. To estimate significant thresholds, the generated TSs were divided by those obtained by 1,000 random permutations of the data performed during SIM analysis. Corrected TSs (cTSs) were obtained. When cTS was higher than 1, the QTL was accepted (type-I error rate below 5). As in Tinker et al. (1996), when evidence for a QTL main effect and a QTL×E interaction were found near the same position, a single QTL was inferred based on the strongest effect.

## Results

### Mean and variation of relative water-content

The environment effect on relative water-content values was highly significant ( $P < 0.0001$ ). Means, minimum and maximum values for the 167 RILs and the two parental lines Tadmor and Er/Apm are presented in Table 2. A significant genotype effect was noticed for M00, F99ir and F99rain. For the two latter environments, the magnitude of extreme values was smaller for the irrigated environment than observed for the rainfed one. The genotype effect was not significant for M01 and T01. Under the field-conditions studied, the difference in RWC between the parental lines was not significant. Considering the rainfed environments, the block effect within each environment was significant, particularly for M01 and T01. The adjusted means generated by fixing the block within the environment effect (Table 2) allows us to reduce this effect. The environment presenting the lowest adjusted mean value for RWC was M00 (64.1%), whereas F99ir but also F99rain have presented the highest means respectively, 94% and 91.5%. The ranges of variation of RWC adjusted means among RILs were 14.87%, 34.94%, 38.22% and 49.40% for F99rain, M00, M01 and T01, respectively. For the latter environment, values varied considerably from 42.95 to 92.35%.

### QTLs for relative water-content

#### *QTL identification in each environment separately*

Table 3 describes the QTLs identified for each environment separately by SIM and/or CIM. A total of six

**Table 2** Means, standard deviations, minimum and maximum values for and relative water-content (RWC), expressed as %, measured in five environments on 167 barley recombinant inbred lines (RILs) issued from the cross between Tadmor and Er/Apm. RIL and block effects are also indicated. Adjusted means generated by fixing the block within environment effect are indicated between

Environment	F99ir# <sup>a</sup> (Montpellier)	F99rain (Montpellier)	M00 (Meknès)	M01 (Meknès)	T01 (Le Kef)
Tadmor	95.92±3.0	90.25±2.6	64.47±11.5	74.40±4.6	69.79±16.7
Er/Apm	97.16±3.1	89.88±4.1	67.42±5.0	64.01±8.0	79.10±13.1
RIL	94.05	91.46 (91.50)	64.60 (64.61)	73.66 (73.61)	74.12 (74.03)
SD	2.51	3.39	7.37	12.26	15.26
Min	88.14	75.63 (82.79)	43.37 (44.65)	32.08 (51.94)	21.83 (42.95)
Max	98.65	100.00 (97.65)	93.82 (79.56)	100.00 (90.16)	98.82 (92.35)
RIL effect	0.0001***	0.0278*	0.0001***	0.5801 ns	0.1779 ns
Block effect	–	0.0385*	0.0472*	0.0001***	0.0001***

\*,\*\*\* Significant at 0.05, 0.001 probability level

<sup>a</sup> # Only one block

parenthesis for the RILs mean, maximum and minimum values of RWC from the four rainfed environments. F99ir: France 1999 with irrigation supply, F99rain: France 1999 rainfed, M00rain: Morocco 2000 rainfed, M01rain: Morocco 2001 rainfed, T01: Tunisia 2001 rainfed

**Table 3** Positions, LODscores and allele effects of QTLs identified for relative water-content in each environment separately with PLABQTL for simple interval mapping (SIM) and composite

interval mapping (CIM). F99ir: France 1999 irrigated, M00rain: Morocco 2000 rainfed, T01: Tunisia 2001 rainfed. Italics: global error between 5 and 10%

Environment	SIM				CIM			
	Chr. Pos.	LODscore	R <sup>2</sup>	Additive effect*	Chr. Pos.	LODscore	R <sup>2</sup>	Additive effect*
F99ir	–	–	–	–	5H McaaEaccS+8	2.79	15.0	+1.492
M00rain	2H Bmag0378+12	2.56	6.8	+2.254	–	–	–	–
	6H BCD348B+7	3.65	9.6	–2.072	6H BCD348B+5	2.88	7.6	–1.657
	6H BCD1+23	3.17	9.0	+2.162	6H BCD1+21	2.80	8.0	+1.918
	5H dhn1+9	4.42	11.5	+2.597	5H SB734B+16	4.18	10.9	+2.133
T01rain	7H A19.4+24	2.61	6.9	–3.346	–	–	–	–

\* Genetic effect expressed as the contribution of allele of Tadmor compared to Er/Apm

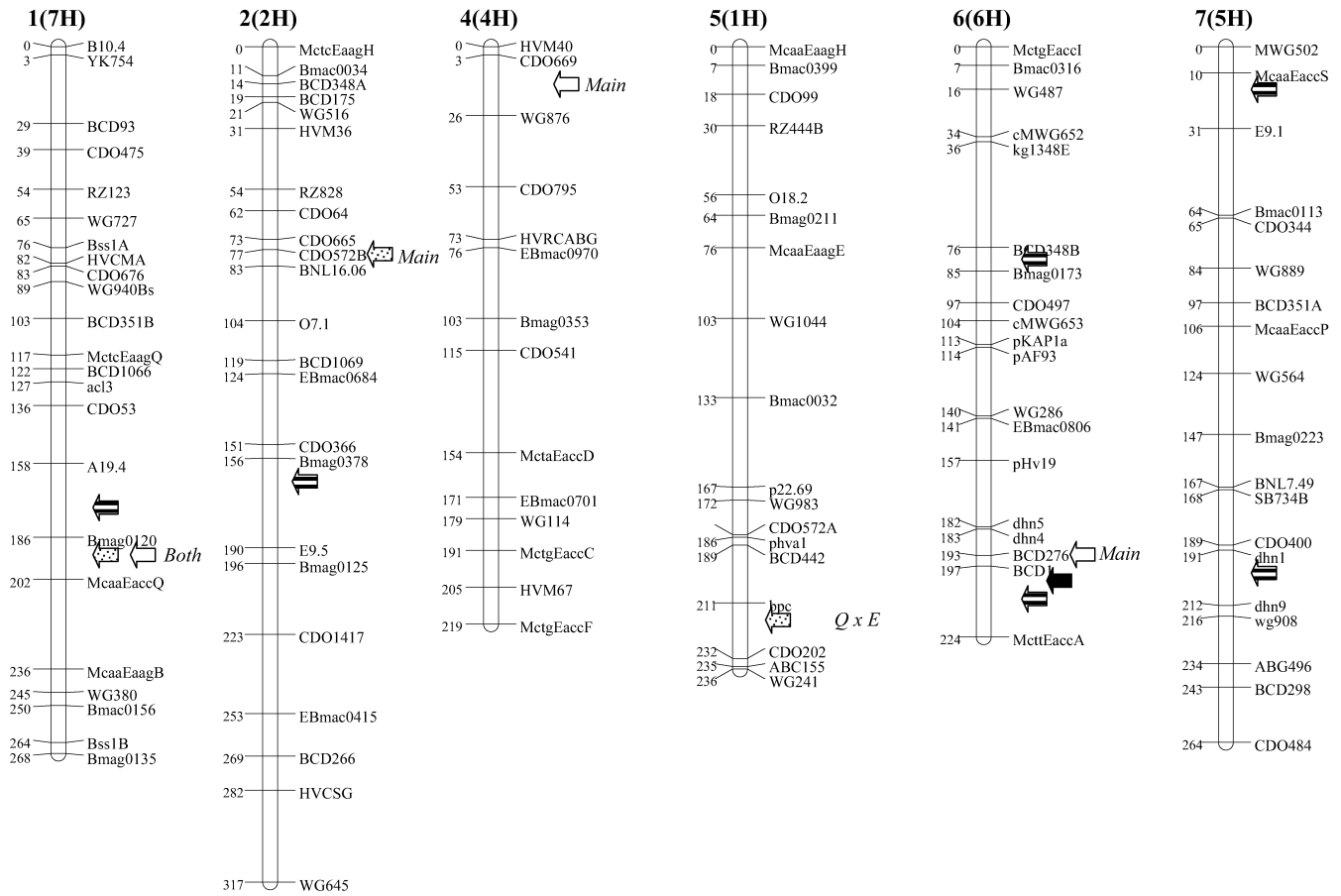
**Table 4** Positions, corrected statistic tests (cTS) and allele effects of QTLs identified for relative water-content across four rainfed environments (E) (A) and all E (B) with MQTL for simplified composite interval mapping (sCIM)

Set of environments	Chr. position	Main effect cTS	QTL×E effect cTS	Allele effect
A	7H Bmag0120+3	1.02	1.28	0.895 E
	2H CDO572B+2	1.15	–	0.995 E
	4H CDO669+4	1.30	–	1.095 T
	1H bpc+16	–	1.30	0.235 E
B	7H Bmag0120+3	1.10	1.28	1.115 E
	4H CDO669+6	1.46	–	0.865 T
	1H bpc+16	–	1.303	0.180 E
	6H BCD276+2	1.06	–	0.880 T

different QTLs were found for three of the fifth studied environments. No QTL was detected for both F99rain and M01 whatever the method employed, whereas one QTL was identified for F99ir, four for M00rain and one for T01rain. For F99ir, the QTL was only detected by CIM on chromosome 5H. For M00rain, the QTL identified on chromosome 2H was only detected by SIM, whereas the three others were detected by both methods and presented a higher LOD-score. They were identified in two areas of chromosome 6H, nearby BCD348B and BCD1, and nearby the dhn1 locus on chromosome 5H. A QTL was identified by only SIM for T01 on chromosome 7H. Tadmor alleles contributed to positive effects for four of the six QTLs identified.

#### *QTL stability across environments for relative water-content*

The QTLs identified as presenting main effects across environments or/and interacting with environments using MQTL with sCIM analysis are presented in Table 4. When considering the four rainfed environments, four QTLs were detected. One QTL presented both effects and was located on chromosome 7H. Two QTLs presented the main-effect across environments. They were located on chromosomes 2H and 4H. The last QTL presented QTL × environment effect and was located on the long arm of chromosome 1H. When the analysis was extended to the five environments studied, four QTLs were detected.



**Fig. 1** QTLs for relative water-content that have been identified with the recombinant inbred line progeny Tadmor × Er/Apm. Lined, dotted, white and black arrows are respectively for QTLs detected in one environment, QTLs presenting QTL × Environment

Among them, three were identified when the analysis was performed on the four rainfed environments and with the same type of effects. These were those mapped on chromosomes 7H, 4H and 1H. The QTL previously found on chromosome 2H was not detected with the additional environment included in the analysis. By contrast another QTL was detected; it presented main effects across environments and was mapped on the long arm of chromosome 6H. The latter QTL was also detected by CIM using PlabQTL (software for QTL analyses) and adjusted entry means generated by fixing the temperature effect (LOD-score of 3.03). Tadmor contributed to positive effects for two QTLs on chromosomes 4H and 6H, whereas ER/APM gave positive effects on the three other chromosomal regions.

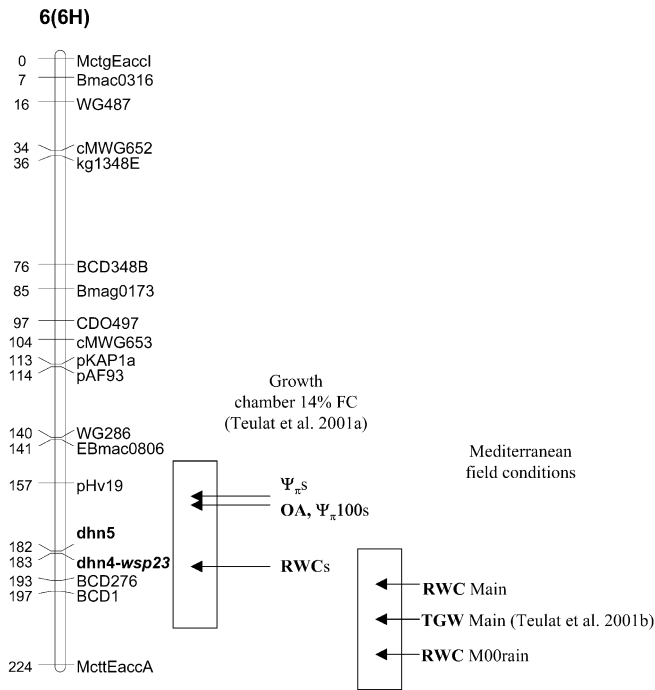
## Discussion

### QTL for relative water content

The QTL results obtained in the present study underlined that several putative genomic regions contribute to the total

interaction, QTLs presenting a main effect across environments and QTLs detected with adjusted entry means generated by fixing the temperature effect

variation of RWC (Fig. 1). The first results obtained with the same barley population grown under controlled conditions at two different soil-moisture contents have also revealed several loci involved (Teulat et al. 2001a). This also agreed with the results from Schonfeld et al. (1988), who have shown that the phenotypic distribution of RWC in F2 and BC indicated that the trait was quantitatively inherited and not controlled by one or two genes in wheat. In rice, Courtois et al. (2000) identified 11 QTLs grouped on nine genomic regions for RWC measured in two different environments, and Price et al. (2002) identified eight QTLs for RWC measured in three different environments. Among the nine genomic areas identified in the present study, two presented QTL×environment interaction (on the long arms of chromosomes 7H and 1H) and four were detected for only one of the environments studied. By contrast, three QTLs presented main effects across four or/and five environments and could be considered as stable regions controlling RWC (chromosomes 2H, 4H and 6H). In addition, when combining the QTL analysis conducted for traits related to plant water-status and/or osmotic adjustment (OA) in a growth chamber (Teulat et al. 2001a) with the present study from field experiments, and



**Fig. 2** Barley chromosome 6H, where several overlapping QTLs were identified for traits related to water-status and drought-tolerance. *RWC*: relative water-content;  $\psi_{\pi}$ : osmotic potential;  $\psi_{\pi 100}$ : osmotic potential at full turgor, osmotic adjustment; *TGW*: thousand grain weight. *Main*: main effect across environments; *M00rain*: Morocco 2000 rainfed environment; *FC*: field capacity

considering the confidence interval of QTL position, common QTL regions were identified. One example is the genomic region of chromosome 2H nearby Bmag0378 that controls RWC, but also several traits related to OA measured under controlled conditions. A QTL controlling RWC for the M00 trial was mapped in the same area. The most-consistent example is the genomic region on the long arm of chromosome 6H (Fig. 2). It 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 (Teulat et al. 1998, 2001a). Recently, the homoeologous region of rice chromosome 2 was also identified as involved in OA (Zhang et al. 2001). Similarly Price et al. (2002) identified a QTL for RWC on rice chromosome 8 that co-localised with a QTL for OA identified in another population (Lilley et al. 1996). This region is homoeologous to a barley region nearby CDO673 where a QTL for RWC was identified in stressed conditions (Teulat et al. 1998). The OA capacity allows cell-turgor maintenance and turgor-dependent processes (Turner and Jones 1980), and RWC is an indicator of the cell volume. These traits are involved directly or indirectly in plant water and turgor status. The genomic region of the barley chromosome 6H was again identified as controlling RWC measured in Mediterranean field conditions. In addition, this is probably the most-stable and confident QTL obtained across the field environments studied (Table 4 and Fig. 1). For the most-stable regions, the nearest molecular markers

could be identified and used to improve breeding efficiency, as a selection criterion for the trait.

### *Drought-tolerance trait evaluation and QTL value for breeding purposes*

Most of the drought-tolerance traits are quantitative. These are difficult to measure on a large number of plants and segregant lines. The difficulty increases when the trait must be evaluated in field conditions. Indeed the genetic part of the phenotypic variation is often hidden due to the abiotic or biotic source of variability acting on the trait (disease attack, risk of inappropriate rainfalls), involving difficulty of trial management and relevant measurement time. In addition, the trait must be measured instantaneously on all the plants, which is nearly impossible. In parallel, it is now commonly accepted that the use of the molecular-genetics approach and of molecular markers could help to improve the selection efficiency. For all those reasons, QTLs for traits evaluating plant water-status and/or osmotic adjustment were previously investigated with barley segregant lines grown under controlled conditions (Teulat et al. 1998, 2001a). However, Price and Courtois (1999) have underlined that locating QTLs for drought resistance mechanisms, by the use of controlled greenhouse or growth room experiments combined with field evaluations under relevant conditions, should allow us to identify QTLs of value for breeding. In the previous experiment conducted under controlled conditions, 13 chromosomal regions were identified as controlling traits related to plant water-status and/or osmotic adjustment with the same genetic background (Teulat et al. 2001a). Considering the difficulty of quantitative trait evaluation under field conditions, the measurements were restricted to relative water-content. However, to assess the variation in the trait and in the QTLs across different drought situations, the trait was measured under several Mediterranean field conditions to confirm, or not to confirm, the QTLs previously identified from the experiment conducted under controlled conditions. The compilation of the data from the two sets of experiments allow us to identify at least three genomic area common from both types of experiments, confirming the interest of the strategy undertaken. These regions seemed to be relevant targets for breeding purposes. The one on the long arm of chromosome 6H was also shown to control thousand-grain-weight across several Mediterranean environments (Teulat et al. 2001b), reinforcing its interest; QTL for a drought-tolerance mechanism, or a criterion being of little value, cannot be shown to improve or stabilise yield under stress conditions or if it causes a substantial reduction of yield under ideal conditions (Price and Courtois 1999). In maize, encouraging results of molecular-assisted-selection under drought conditions were obtained (Ribaut et al. 1999).

The influence of the environment was also noticed when data analyses have shown no significant genotype effect for RWC in some environments studied (Table 2).

As an example, the difference in RWC between the two parental lines Tadmor and Er/Apm was not significant under the field-conditions studied. By contrast, the two parental lines have presented high significant differences for the same trait previously measured under controlled conditions (Teulat et al. 1997a). Tadmor, considered as the drought-tolerant genotype in terms of yield stability, has presented higher RWC values compared to Er/Apm at different soil-moisture contents during an imposed water deficit (Teulat et al. 1997a). The large differences observed for RWC in the RIL population could be due to differences in solute accumulation and osmotic adjustment, the two traits characterising the population and the two parental lines studied (Teulat et al. 1997a, 2001a). The use of adjusted entry means, generated by fixing the block within the environment effect, has improved the power of QTL detection. This also underlined the need of relevant experimental designs in this type of strategy.

#### *Which genes under the RWC variation?*

The molecular genetics approach could also help our understanding of the process of drought-tolerance through genetic interaction between traits or co-locations of QTLs with gene sequences. It is now known that the grass genomes contain gene-rich compartments (Sandhu and Gill 2002). This has an effect on recombination that was shown to be high in gene-rich barley regions (Kunzel et al. 2000). This also shows the difficulty to identify the genes that are really involved in an individual-trait phenotypic variation. However, the co-locations of QTLs controlling water-status and/or turgor with sequences corresponding to *dhn* genes on the same portion of chromosome 6H, was a great indication of the possible role of these genes in the variation of plant water-status under drought (Fig. 2). The latter region of chromosome 6H was previously proved to contain a cluster of *dhn* genes including the barley *dhn4* and *dhn5* (Campbell and Close 1997), whereas the wheat *wsp23* sequence corresponds to an analog of barley and maize dehydrin protein, and to rice and wheat RAB proteins expressed under water stress (Joshi et al. 1992). Another chromosomal region contains a QTL for RWC for M00rain and a dehydrin locus *dhn1* on chromosome 5H QTL. The dehydrins are water-soluble lipid-associated proteins that accumulate in response to dehydration, low temperature, osmotic stress or ABA, or during seed maturation (Close et al. 1989). Several QTLs controlling tolerance traits, and particularly freezing tolerance, have already been identified close to dehydrin genes (Campbell and Close 1997). These authors have underlined that the recurring physiological and genetic correlations constitute mounting evidence that dehydrin genes may be key genetic determinants of stress tolerance in a number of species, particularly freezing and drought-tolerance. The first example was for a QTL for winter-hardiness overlapping a cluster of *dhn* genes, including *dhn1* on barley chromosome 5H associated with a cold-specific induc-

tion of a member of this dehydrin family (Pan et al. 1994; Van Zee et al. 1995). The emergence of a biochemical role of these proteins shows that they could preserve structural integrity, particularly membrane integrity, by inhibiting the coagulation of macromolecules (Close 1996, 1997). Recently Koag et al. (2003) have shown the binding of maize DHN1 to lipid vesicles, suggesting membrane stabilisation under stress conditions. The link between cell volume/turgor maintenance and the properties of these proteins seems possible but must be proved. The positional cloning of the main QTL and the allelic variation study of *dhn* genes in a collection of barley genetic resources differing for their drought-tolerance response, could elucidate if the *dhn* genes are involved in plant water-status and drought-tolerance variation. Ismail et al. (1999) have conducted this type of experiment on *Vigna unguiculata* plantlets. They have demonstrated the co-segregation of a dehydrin gene with chilling tolerance, and the usefulness of the normal protein compared to a dehydrin mutant-allele in this phenomenon.

#### Conclusion

Nine chromosomal regions were found as controlling relative water content in Mediterranean field-grown barley. Among them, at least four could be considered as stable regions across several growth conditions, because they presented main effects across several field environments or/and because they are the same as the regions previously identified under controlled conditions. The validation of a part of the QTL identified from a controlled condition experiment demonstrated the interest of this type of experiment to discover the potential chromosomal area involved in the genetic control of a quantitative trait, limiting the environment influence. The first one allows us to better measure the genetic part of the phenotypic variation by limiting environment effects; the second one represents the agronomic conditions. When comparing the present results with previous ones obtained on the same genetic background (Teulat et al. 1998, 2001a, b, 2002), one QTL area on the long arm of chromosome 6H was confirmed to be of particular interest for cereal-breeding purposes under drought. In addition, the presence of dehydrin genes increases the interest for its physical mapping to identify the most-suitable allele for marker-assisted selection and to confirm, or not to confirm, the role of dehydrins in relative water-content variation.

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