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Several QTLs involved in osmotic-adjustment trait variation in barley (*Hordeum vulgare* L.)

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Abstract Osmotic adjustment (OA) was previously demonstrated to be an important adaptive mechanism of drought tolerance in cereals. In order to determine which genomic regions are involved in OA variation, 187 barley (*Hordeum vulgare* L.) recombinant inbred lines (RILs) derived from a cross between Tadmor (drought tolerant) and Er/Apm (susceptible) were studied in a growth chamber for their OA capacity (through correlated traits and by calculation), at an early growth stage and under two water treatments (soil moisture of 14% and 100% of field capacity). The continuous distribution of the traits and their broad-sense line heritabilities, ranging from 0.04 to 0.44, indicated that OA and related traits should have a polygenic nature. A subset of 167 RILs were also genotyped using 78 RFLP, 32 RAPD and three morphological markers and a linkage map was constructed. Despite strong environmental effects acting on the traits, interval mapping and single-marker ANOVA allowed the detection of three QTLs for relative water content (RWC), four QTLs for osmotic potential (ψ_π), two QTLs of osmotic potential at full turgor ($\psi_\pi 100$) and one QTL for osmotic adjustment at a soil moisture of 14% field capacity. For the irrigated treatment, only two QTLs were detected: one for RWC and one for $\psi_\pi 100$. Two chromosomal regions were involved in several OA-related trait variations and could be considered as regions controlling OA; these were present on chromosome 1 (7H) and chromosome 6 (6H), whereas other regions were specific for one trait. No major QTL was found. However, the genomic region involved in OA-related traits on chromosome 1 (7H) in barley seemed to be conserved for OA variation among cereals. Epistatic effects, with or without additive effects, acted on the traits.

Key words Water stress · Barley · Osmotic adjustment · Adaptation · QTL · Synteny

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Introduction

Barley is a major crop cereal, showing a wide range of adaptation to various environments. Drought is an important abiotic factor limiting yield, and barley seems to be relatively well adapted to water deficit (Ceccarelli 1987). This species could thus be a good model to understand drought and water-stress tolerance mechanisms. There are few traits considered to be important for adaptation to drought. Osmotic adjustment (OA) has been identified as a major trait of interest, and is associated with the whole-plant response to water stress.

Osmotic adjustment is defined as a decrease of osmotic potential within the cells, due to solute accumulation during a period of declining leaf water potential (Ludlow and Muchow 1990). At low soil moisture, OA maintains cell turgor. In several crop species, including wheat and sorghum, it has been shown that OA not only permits survival and maintenance of vital processes, but also contributes to increase yield and yield stability under drought (Morgan 1983; Santamaria et al. 1990). Acevedo (1987), Grumet et al. (1987) and Blum (1989) have found that some barley genotypes showed a constitutive low osmotic potential: the

osmotic potential was low at full turgor in plants not previously exposed to drought. Grumet et al. (1987) showed that barley populations with a greater constitutive osmotic capacity had a lower yield than those that had a lower capacity. Therefore a distinction between these two osmotic phenomena has to be considered in barley.

Molecular markers offer a promising tool to understand the genetic control of drought tolerance as well as to follow the introduction of important genomic regions for tolerance into susceptible genotypes by marker-assisted selection (MAS). Large variability has been demonstrated in several species for OA, but this trait is difficult to manipulate in genetic studies (Morgan 1983; Blum 1989). In cereals, some authors have proposed methodological protocols for the evaluation of OA on a large number of plants (James and Lilley 1997; Teulat et al., unpublished results). These methods could be applied to segregating populations to identify the quantitative trait loci (QTLs) responsible for the variation of OA.

Many genes may be involved in tolerance mechanisms and the identification of several QTLs involved in drought- or dehydration-tolerance trait variation in rice (Lilley et al. 1996) or in maize (Lebreton et al. 1995) have confirmed their polygenic nature. Furthermore, several modifications of gene expression were observed under water stress (Close et al. 1989; Bray 1997). The expression of stress-induced genes is an essential part of tolerance mechanisms; but for many of these genes no direct function in tolerance has been clearly demonstrated. Some of the QTLs involved in drought tolerance may correspond to such genes acting directly or in regulatory functions. Consequently, the location of these candidate genes near or within QTLs involved in adaptation could give some information on their role.

A population of 187 barley recombinant inbred lines (RILs) was used to study the genetics of OA in a growth-chamber experiment under water-stress and well-watered (control) conditions. A subset of 167 RILs was used for the construction of a genetic map using RFLP and RAPD markers, including some candidate genes for abiotic stress tolerance as RFLP probes. The objectives of this study were: (1) to identify key chromosomal regions involved in osmotic-adjustment variation, (2) to compare these regions with those already identified in cereals, i.e. wheat (Morgan 1991) and rice (Lilley et al. 1996), and (3) to examine the feasibility of a marker-assisted selection for OA in barley.

Materials and methods

Genetic material

A segregating population of F_8 recombinant inbred lines (RILs) derived from the cross between Tadmor and Er/Apm was employed. Tadmor is a two-row variety selected from a Syrian landrace which is very well adapted to the dry conditions (250–400 mm rainfall) of the Middle-East and is characterized by a high yield stability (Grando 1989) and a high OA capacity (Teulat et al. 1997b). Er/Apm is a two-row improved variety developed by ICARDA. It is adapted to moderate rainfall conditions (Acevedo 1987) and also has a constitutive osmotic capacity (Acevedo and Naji 1986; Teulat et al. 1997b). Recombinant inbred lines were advanced by bulk until the F_4 generation at ICARDA (Syria) and then by single-seed descent until the F_8 generation at CIMMYT (Mexico).

Controlled conditions experiment

A total of 187 RILs and the two parents was used in a balanced incomplete-block random design. It contained nine incomplete experiments (nine blocks), performed chronologically from October 1994 to February 1996. In each block, a water-stress treatment and an irrigated treatment were conducted. The nine blocks were necessary to study five replicates (five different plants) per RIL and per water treatment, taking care that two replicates of the same line were never included in the same block. Nevertheless, any time a plant was present in the water-stress treatment, another plant of the same line was present in the irrigated treatment of the same block. Each block included 105 RILs and 21 pots per treatment. Each pot contained plants of five different RILs and, for each of these plants, neighbors were drawn at random and were never similar. Pot locations were re-randomized within each block every 3rd day.

The plants were initially grown in a greenhouse at ENSAM-INRA (France) in plastic pots (2.5 l) containing compost and vermiculite (3 : 1; v/v) until the three-leaf stage (15-h photoperiod with relative humidity 70–80% and a light intensity of 85 W/m; a 20°C day and a 15°C night, temperature) and then transferred to a growth chamber (Conviron E25). Experimental conditions in the growth chamber were quoted as: 15-h photoperiod with a photon flux density of 450 μmol m^{-2} s^{-1}, 22°C day, 20°C night, relative humidity 70% day, 75% night (programmed values). Fertiliser (500 mg/pot of a mix of 15% N, 29% SO_3, 10% P_2O_5, 15% K_2O and 2% MgO solution Hakaphos BASF) was applied once a week before the beginning of the stress. The field capacity of the soil was evaluated by weighing pots one day after complete re-hydration. Water stress was applied at the four-leaf stage by withholding water. Twelve days of water deficit were necessary to reach a soil moisture of 14% field capacity. Measurements were recorded at that soil moisture for the stressed plants and at a soil moisture of 100% field capacity for the irrigated plants grown during the same period. Osmotic adjustment was first evaluated through correlated traits (Teulat et al. 1997b). Leaf relative water content (RWC) was measured on the last fully expanded leaf according to Barrs and Weatherley (1962) as RWC (%) = (FW − DW) × 100/(TW − DW), where FW is the leaf fresh weight, TW the turgid weight, and DW the dry weight. To evaluate leaf osmotic potential (ψ_π) values, the penultimate leaf was cut, wrapped in aluminium foil, frozen in liquid nitrogen and stored at −20°C until measurement. Osmotic potential was measured using a freezing point micro-osmometer (Roebling 13GS/IS, Germany). RWC and ψ_π were used to calculate the leaf osmotic potential at full turgor ($\psi_\pi 100$; Wilson et al. 1979) as $\psi_\pi 100 = \psi_\pi \times (\mathrm{RWC} - \mathrm{B})/(100 - \mathrm{B})$ where B is the apoplastic water (Tetlow and Farrar 1993). In order to avoid the confusion between osmotic adjustment as a real adaptive trait and the constitutive osmotic capacity, OA was then calculated according to Ludlow et al. (1983) as $\mathrm{OA} = \psi_\pi^s 100 - \psi_\pi^c 100$ where $\psi_\pi^s 100$ is the $\psi_\pi 100$ of the stressed sample and $\psi_\pi^c 100$ is the $\psi_\pi 100$ of the well-watered control sample.

Standard analyze of variance of RWC, ψ_π, $\psi_\pi 100$ and OA were performed considering block and pot within-block effects as fixed effects using the GLM SAS procedure (SAS Institute, Cary, N.C.) to determine if differences between lines were evident for each trait.

Table 1 Candidate genes mapped. ABA = Abscisc acid, GA = Gibberellic acid

Clone	Protein	Species	Induction	References
HVA1	LEA 3 protein group	Barley	ABA, cold, heat-shock and drought	Hong et al. (1988) Sutton et al. (1992)
Dhn1 Dhn2 Dhn4	LEA 2 protein group-dehydrins	Barley	ABA, drought	Close et al. (1989)
wsp23	Analog to barley and maize dehydrin protein and to rice and wheat RAB proteins	Wheat	Water stress	Joshi et al. (1992)
KG1348	Full-length thionin	Barley	Jasmonate	Gausing (1987)
pAF93		Barley	Cold and drought	Cattivelli and Bartels (1990) Grossi et al. (1992)
p22_69	Aldose reductase	Barley	Freezing, NaCl, ABA, GA and drought	Bartels et al. (1991)
Ss1	Sucrose synthase	Barley	Cold and anaerobiosis	Sanchez de la Hoz et al. (1992)
Acl3	Acyl carrier protein III	Barley	Seems to be constitutively expressed	Hansen and von Wettstein-Knowles (1991)

Adjusted means were obtained by regression on block and pot within-block effects by the least-squares method. Components of variance were then computed using the mean-squares expectation obtained from the VARCOMP SAS procedure (Scheffe 1959). Estimates of the variance components σ^2 g (genotypic variance) and σ^2e (error variance) allowed the calculation of the broad-sense line-heritabilities of the mean of lines (h^2) for all the traits as $h^2_{line} = \sigma^2 g/(\sigma^2 g + \sigma^2 e/k)$, where k is the number of replicates per line.

Construction of the genetic map

Two hundred and ninety nine clones from different sources, including anonymous cDNA and genomic clones of barley (BCD, MWG and cMWG, ABG and ABC), wheat (WG) and oat (CDO) as well as cDNA of known function and candidate genes, were used for polymorphism screening. The clones corresponding to the genes which revealed polymorphism are described in Table 1.

To evaluate polymorphism between Tadmor and Er/Apm, DNA was extracted from young leaves isolated from several plants per line for one part in CIMMYT (Mexico) using the method of Hoisington et al. (1994) and for another part at ENSAM-INRA (France), initially using the method of Roger and Bendich (1985) with minor modifications, and then the method of Saghai-Maroof et al. (1984) modified by Hoisington et al. (1994). The RFLP procedure used at CIMMYT for parental screening followed the non-radioactive protocols described in Hoisington et al. (1994) with five restriction endonucleases used separately (*Eco*RI, *Eco*RV, *Bam*HI, *Hin*dIII, *Dra*I). At ENSAM-INRA, blots were prepared after digestion with six restriction enzymes separately (the same as those used in CIMMYT and *Sst*I). The restriction fragments were separated by electrophoresis on 0.8% agarose gels in TAE 1 × buffer and then transferred onto nylon blots (Hybond N+, Amersham, UK) under alkaline conditions (0.4 N NaOH). Probes were labelled with ^{32}P-αdCTP using the Amersham Megaprime commercial kit (RPN 1604/5/6/7). Pre-hybridization and hybridization were performed according to Hoisington et al. (1994). Blots were then washed rapidly in 2 × SSC, 0.1% SDS at room temperature, and for 15 min twice in 2 × SSC, 0.1% SDS at 65°C, and twice in 1 × SSC, 0.1% SDS at 65°C.

For map construction, DNA was isolated from several plants per line grown in a greenhouse at INRA (Clermont-Ferrand, France) and in the field at ENSAM-INRA (Montpellier, France). The DNA extraction and RFLP procedures used to screen the RFLP markers on the 167 RILs in Clermont-Ferrand were done according to Lu et al. (1994) and as previously described at ENSAM.

In addition, 85 RAPD decamer primers from Bioprobe were screened on the two parents. PCR amplification conditions were performed in a final volume of 25 µl containing 10 × Appligene buffer, 0.5 µM primer, 0.2 mM of each dNTP, 0.4 units of *Taq* polymerase (Appligene) and 150 ng of genomic DNA. Amplifications were carried out using a Biometra (Eurogentec, France) thermocycler with an initial denaturation step of 4 min at 94°C, 35 cycles of amplification of 1 min at 94°C, 1 min at 38°C and 1 min at 72°C, followed by a terminal extension step of 6 min at 72°C. Amplification products were analyzed after electrophoresis in 1.5% TAE agarose gels and ethidium bromide staining. Thirty two reproducible amplified fragments were screened on the RILs with the same procedure. Black pericarp color (lpc), the presence of anthocyanin on the stems (anth) and glaucousness (glau) notations, done in the field at Montpellier (France) in 1995 and at Granada (Spain) in 1996, were also used as morphological markers and scored as dominant markers.

A total of 78 RFLP, 32 RAPD and three morphological markers were used for map construction. Segregation of alleles at each locus was checked against the expected ratio (1:1 for a RIL population) by using a chi-square test with a significance level of 1%. The linkage map was constructed using the Haldane (1919) mapping function with MAPMAKER version 3.0b (Lander et al. 1987) assuming the data were collected on RILs. A minimum LOD threshold of 3.0 and a maximum recombination fraction of 0.50 were used.

Detection of quantitative trait loci

Associations between genetic markers and traits were detected by interval mapping using MAPMAKER/QTL version 1 (Lander and Botstein 1989) and supported by a single-marker analysis of variance using the ANOVA SAS procedure. The normal distributions of phenotypic adjusted means were tested for all traits prior to analysis. No deviations from normality were observed for any trait. Although a minimum LOD score of 2.4 can be considered significant based on the criteria described by Lander and Botstein (1989), putative QTLs detected when the LOD score $\geq$2.0 were included, considering that high environmental effects could have influenced the power of detection of the QTLs. In order to look for additional marker-trait associations with minor effects, another analysis was conducted with all the major QTLs as fixed factors separately. The analysis of variance was then carried out with $Pij = \mu + Mi + Eij$, where Pij is

the mean trait of the j genotype having the i marker, Mi the marker i effect, μ the mean trait of all the RILs and Eij the residual. Marker-loci effects were declared significant when the probability levels associated with *F*-values were less than 0.005. The coefficient of determination (R^2) is an estimate of the proportion of the phenotypic variation accounted for by the model. Dominance effects could not be detected because of the genetic structure of the population (RILs; no heterozygous class contributed to the LOD). In order to identify the regions acting in epistasis, a 2nd order analysis of variance was carried out to detect interactions between all pairs of markers with the following model $Pijk = \mu + Mi + Mj + (Mi \times Mj) + Eijk$, where Pijk is the mean of the kth genotype having the i and j markers, Mi and Mj the marker i and j effects respectively, $Mi \times Mj$ the marker i and j interaction effect and Eijk the residual. In this model, the explained percentage of trait phenotypic variation was evaluated as $R^2 = SS_M/(SS_M + SS_R)$, where SS_M is the sum of squares of the marker combination and SS_R is the sum of squares of the residual. The interaction was declared significant at $P < 0.001$.

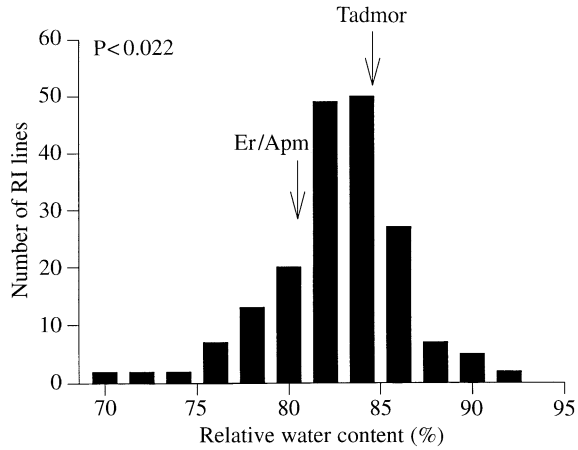

Fig. 1 Distribution of adjusted means of 167 recombinant inbred lines for relative water content (RWC) at a soil moisture of 14% field capacity. Parental values are indicated by *arrows*. *P* is the significance level in ANOVA of the line effect on RWC

Results

Expression of physiological traits

The analysis of variance revealed significant differences between the two water treatments ($P < 0.0001$) for RWC, ψ_π and $\psi_\pi 100$. The adjusted means of RWC decreased from 98.4 to 82.3%, ψ_π decreased from -1.15 to -1.61 MPa, and $\psi_\pi 100$ decreased from -1.13 to -1.20 MPa between the two treatments. Significant differences between the blocks ($P < 0.0001$) were also identified within each treatment. For example, in the water-stress treatment, the adjusted means of RWC, ψ_π and $\psi_\pi 100$, significantly increased linearly with time indicating that, despite all measurements having been carried out at 14% field capacity, there was an uncontrolled chronological evolution of the total adjusted means of all the traits between successive blocks which could partially explain the block effects. Comparing the adjusted means of each block for all the traits with a Duncan's test (data not shown), we noticed that the plants in the first four blocks were more affected by the stress and that they were more heterogeneous than the five last ones, even though soil moisture was controlled every day. As this result could not be explained by variations in field capacity, it could have resulted from an uncontrolled decrease of the photon flux density or an increase of the relative air humidity in the growth chamber (despite the fact that the programmed experimental conditions were supposed to be the same in each block). Consequently, all the analyses were performed on the total experiment (nine blocks and 187 RILs) and on two sub-groups: a three-replicate minimum per line in the four first blocks (85 RILs) and in the five last blocks (105 RILs) to examine the responses of the plants to water stress as a function of the stress intensity.

Figure 1 illustrates the frequency distribution for RWC adjusted means among 187 RILs (nine blocks) at 14% field capacity. The RILs displayed an adjusted mean for RWC of 82.3% ranging between 70.0 and 92.0%. Significant line differences were observed for this trait ($P < 0.022$) and Tadmor had a higher RWC than Er/Apm (Table 2). In the irrigated treatment, RWC had an adjusted mean of 98.4%. Due to a high intra-line variation for ψ_π and $\psi_\pi 100$ in the water-stress treatment and for OA, no significant line differences were observed for these traits even in the five last blocks: $P < 0.13$, $P < 0.85$ and $P < 0.08$ respectively. All the trait distributions were continuous in the two water treatments showing their quantitative nature. Figure 2 shows the frequency distribution for OA among 105 lines (last five blocks). The most-negative values represent high osmotic adjustment in this cross ($OA = \psi_\pi^s 100 - \psi_\pi^c 100$). Tadmor had a higher OA capacity than Er/Apm, and Er/Apm showed a constitutive osmotic capacity (lowest osmotic potential at full turgor without stress) (Fig. 2, Table 3). However, the values of the two parents for all the traits were not very different (Tables 2, 3). A large variation was noted for the broad-sense line heritabilities found in this study ranging from 0.04 for RWC in the irrigated treatment to 0.44 for RWC in the first four blocks (85 RILs) in the water-stress treatment (Table 2). The heritability of RWC was higher at 14% field capacity than for the irrigated treatment. However, the high environmental effects observed decreased the heritabilities of all the traits (Tables 2, 3). For example the genotypic variance of RWC in the total experiment was 2.72 whereas the environmental variance reached 38.53. In the last five blocks, the heritability of OA was 0.20.

Genetic marker segregation and linkage map

Of the 299 RFLP probes used, 41.8% showed a polymorphism between the parental lines with at least one

Table 2 Adjusted means, variance components and broad-sense line heritabilities (h^2_{line}) for osmotic-adjustment related traits obtained from the water-stressed and irrigated RIL data of the total experiment (9 blocks and 187 RILs), of the first four blocks (85 RILs) and of the last five blocks (105 RILs). RWC: Relative water content measured in %; ψ_π: leaf osmotic potential and $\psi_\pi 100$: leaf osmotic potential at full turgor measured in Mega-Pascals (MPa). #: mean of the effective number of replicates per line used for the determination of the heritability of each trait. σ^2g: genotypic variance; σ^2e: environmental variance

	RWC (%)			ψ_π (MPa)			$\psi_\pi 100$ (MPa)		
Water-stress treatment									
Number of lines studied	187	85[a]	105	187	85[a]	105	187	85[a]	105
Adjusted means									
Tadmor	83.79	–	92.76	−1.58	–	−1.23	−1.23	–	−1.11
Er/Apm	81.64	–	92.34	−1.56	–	−1.17	−1.19	–	−1.02
F8 RILs	82.34 ± 6.25	69.66 ± 6.66	93.88 ± 2.29	−1.61 ± 0.24	−2.11 ± 0.32	−1.21 ± 1.14	−1.20 ± 0.15	−1.33 ± 0.19	−1.10 ± 0.11
Variance components									
σ^2g	2.72	11.94	1.105	0.00038	0.0076	0.0014	0.0014	0.004	0.0015
σ^2e	38.53	44.34	5.229	0.060	0.1005	0.02	0.0224	0.036	0.0125
#RIL replicates	3.87	2.86	2.42	3.64	2.72	2.45	3.63	2.49	2.44
h^2_{line}	0.22	0.44	0.34	0.02	0.05	0.15	0.18	0.21	0.23
Irrigated treatment									
Number of lines studied	187	85[a]	105	187	85[a]	105	187	85[a]	105
Adjusted means									
Tadmor	99.19	–	99.08	−1.09	–	−1.16	−1.09	–	−1.14
Er/Apm	97.62	–	100.1	−1.17	–	−1.11	−1.14	–	−1.11
F8 RILs	98.35 ± 1.75	97.05 ± 2.25	99.4 ± 1.1	−1.15 ± 0.11	−1.19 ± 0.12	−1.13 ± 0.09	−1.13 ± 0.11	−1.15 ± 0.11	−1.01 ± 0.08
Variance components									
σ^2g	0.033	0.077	0.046	0.00012	0.002	0.0015	0.00019	0.0019	0.0012
σ^2e	3.06	5.060	1.207	0.0119	0.0135	0.008	0.0116	0.013	0.0065
#RIL replicates	3.71	2.60	2.36	3.38	2.44	2.15	3.42	2.43	2.15
h^2_{line}	0.04	0.04	0.08	0.03	0.27	0.29	0.05	0.26	0.28

[a] Parents were not in this subgroup

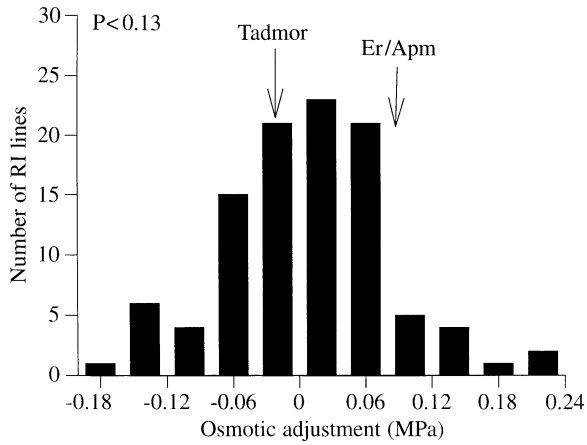

Fig. 2 Distribution of adjusted means of 105 recombinant inbred lines (five last blocks) for osmotic adjustment (OA) at a soil moisture of 14% field capacity. Parental values are indicated by *arrows*. *P* is the significance level in ANOVA of the line effect on OA

enzyme. Sixty five probes revealed single-copy loci, whereas four probes revealed two loci and one probe, the thionin gene KG1348, allowed five different loci to be mapped. About 66% of the RAPD primers revealed a polymorphism between the two parental varieties. The segregation data for 78 polymorphic RFLP markers, 32 RAPD markers, and three morphological markers were scored on 167 RILs. Three RAPD and two RFLP markers displayed segregation distortion at the 1% level and these are indicated on the map (Fig. 3). Seven of the RFLP markers and all the RAPD markers were scored as dominant markers. The linkage map obtained showed 16 linkage groups and 16 markers remained unlinked (eight RFLPs, six RAPDs and two morphological markers). The use of anchor probes permitted us to assign the linkage groups to the barley chromosomes. One linkage group could not be assigned and was named group 8 (Fig. 3). Relaxing the mapping conditions to a minimum LOD score value of 2.8 allowed three unlinked RFLP markers, previously located by comparison to the literature, to be mapped. The final linkage map covered 1056.3 cM with a mean distance between linked markers of 14.5 cM. On several chromosomes, few markers co-segregated (the Acl3 and WG1042 loci on chromosome 1 for example). This genetic map was co-linear with previously reported barley maps (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993). However, the estimated genetic distances between adjacent pairs of some RFLP loci deviated from estimates in other barley maps. The pAF93 locus (Cattivelli and Bartels 1990) was not mapped before and was positioned for the first time on this genetic map on chromosome 6 (6H).

Quantitative trait loci involved in OA variation

The QTLs detected by interval mapping are summarized in Table 4. Several chromosomal regions revealed significant effects. For the water-stress treatment, three regions were identified for RWC: two on chromosome 1 (7H) (region A for the total experiment and B for the first four blocks) and the most important effect on chromosome 6 (6H) (region J for total experiment), which explains 7.4% of the phenotypic variation for the 167 RILs. For each of these genomic regions Tadmor alleles conferred a positive effect on RWC. QTLs involved in ψ_π, $\psi_\pi 100$, and OA were only found using the data from the last five blocks (105 RILs) which were more homogeneous than those of the first four blocks (85 RILs). Two of the four QTLs found for ψ_π were mapped at the same loci as two RWC QTLs on chromosomes 1 (7H) (region A) and 6 (6H) (region J), explaining 12.3% and 24% of the phenotypic variation respectively. Two additional QTLs were found on chromosome 2 (2H), explaining 10.8% and 15.1% of the phenotypic variation (region C and D). For $\psi_\pi 100$, two QTLs were identified on chromosomes 5 (1H) (region F) and 6 (6H) (region J) and explained 17.4% and 26.7% of the phenotypic variation. Only one QTL was noted for OA on

Table 3 Adjusted means, variance components and broad-sense line heritabilities (h^2_{line}) for osmotic adjustment (OA) [evaluated as $OA = \psi^s_\pi 100 - \psi^c_\pi 100$, where $\psi^s_\pi 100$ is the $\psi_\pi 100$ of the stressed sample and $\psi^c_\pi 100$ is the $\psi_\pi 100$ of the well-watered control sample] in the total experiment (9 blocks and 187 RILs), in the first four blocks (85 RILs) and in the last five blocks (105 RILs). #: mean of the effective number of replicates per line used for the determination of the heritability of each trait. $\sigma^2 g$: genotypic variance, $\sigma^2 e$: environmental variance

	OA (MPa)		
Number of lines studied	187	85[a]	105
Adjusted means			
Tadmor	− 0.082	–	− 0.012
Er/Apm	− 0.014	–	0.080
F_8 RILs	0.0021 ± 0.19	− 0.003 ± 0.23	− 0.003 ± 0.15
Variance components			
$\sigma^2 g$	0.0002	0.0052	0.0027
$\sigma^2 e$	0.0354	0.054	0.0210
# RIL replicates	3.15	2.26	1.96
h^2_{line}	0.02	0.18	0.20

[a] Parents were not in this subgroup

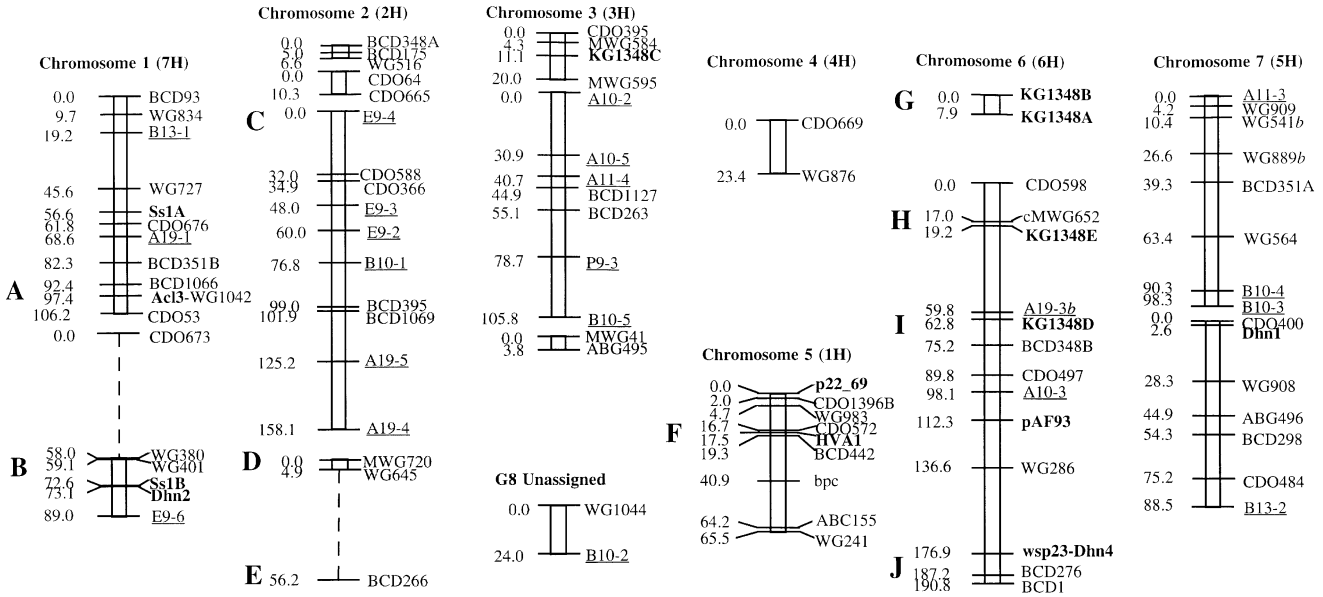

Fig. 3 Barley linkage map of Tadmor × Er/Apm F_8 recombinant inbred lines showing the 16 linkage groups assigned to the barley chromosomes except for group 8. The names of the chromosomes are indicated at the top together with their correspondence in wheat. Loci names are on the right and cumulative distances in centi-Morgans on the left. RAPD markers are *underlined* and known-function gene loci are in *bold letters*. *Hatched lines* are indicated when $d > 0.50$ and when unlinked markers already assigned could be mapped. *b* indicates markers where distortion of the segregation ratio was significant at the 1% level. *Bold letters* on the left of chromosomes indicate the regions where QTLs involved in osmotic-adjustment parameters were detected

chromosome 6 (6H) in the same region as the QTLs found for RWC, ψ_π, and $\psi_\pi 100$ (region J). This QTL was close to the Dhn4 locus and explained 17.7% of the phenotypic variation. For the irrigated treatment, two regions on chromosome 1 (7H) close to the Ss1B locus (region B) and on chromosome 6 (6H) (region H) were respectively found to be implicated in RWC and $\psi_\pi 100$ variation. When major QTLs were fixed, no more additional regions were detected. The analysis conducted by the single-marker method confirmed all the nearest

Table 4 QTLs detected by the interval mapping method. *QTLs detected on the four first blocks, **QTLs detected on the five last blocks. RWC: relative water content, ψ_π: leaf osmotic potential, $\psi_\pi 100$: leaf osmotic potential at full turgor, OA: osmotic adjustment. %Var: individual variance explained by the QTL. Genomic regions referred to in Fig. 3 are also indicated

Traits evaluated in F_8 lines	Chromosome	Marker (distance to peak LOD value in cM)	Genomic region	Estimated genetic additive effects[a]	Magnitude of peak LOD	%Var
Water-stress treatment						
RWC	1	Acl3 (0)	A	1.75	2.09	5.8
	1	WG380* (0)	B	3.29	2.07	8.1
	6	Dhn4 (8)	J	2.01	2.51	7.4
ψ_π	1	Acl3** (0)	A	0.07	2.44	12.3
	2	E9_4** (0)	C	0.07	2.01	10.8
	2	MWG720** (4)	D	0.08	2.27	15.1
	6	WG286** (21)	J	0.10	3.05	24.0
$\psi_\pi 100$	5	BCD442** (4)	F	0.06	2.99	17.4
	6	WG286** (20)	J	0.08	2.23	26.7
Irrigated treatment						
RWC	1	Ss1B (2)	B	− 0.54	2.22	9.1
$\psi_\pi 100$	6	KG1348E (31.8)	H	0.04	2.45	9.2
Water-stress vs irrigated						
OA	6	WG286** (30)	J	0.11	2.61	17.7

[a] Genetic effect expressed as the change in the value of each trait due to the contribution of an allele from Tadmor compared to Er/Apm

Table 5 Effects of marker interactions on osmotic adjustment and osmotic-adjustment traits obtained when $P < 0.001$ for the total experiment. RWC: Relative water content; ψ_π: leaf osmotic potential; $\psi_\pi 100$: leaf osmotic potential at full turgor; OA: osmotic adjustment. R^2: individual variance explained by the interaction of markers on the trait, *P*: probability of significance. T: Tadmor allele; E: Er/Apm allele. TT: combination of two alleles of Tadmor; EE: combination of two alleles of Er/Apm; TE: combination of the Tadmor allele for the first marker and the Er/Apm allele for the second marker; ET: combination of the Er/Apm allele for the first marker and the Tadmor allele for the second marker. No significant interaction was found on the five last blocks. Genomic regions # as referred to in Fig. 3

Traits evaluated in F_8 lines	Markers in interaction	Chromosomes	Genomic regions #	*P*	R^2	Phenotypic mean of SSD lines with alleles of			
						TT	TE	ET	EE
Water-stress treatment									
RWC	BCD1066-BCD1	1-6	A-J	3×10^{-5}	11	82.84	84.31	83.5	80.36%
	BCD1-BCD276	6-6	J-J	7×10^{-5}	10	82.62	83.63	84.76	81.70%
$\psi_\pi 100$	BCD1066-CDO1396A	1-7	A-?	4.7×10^{-4}	9	− 0.76	− 0.74	− 0.72	− 0.77MPa
Irrigated									
ψ_π	BCD1-KG1348D	6-6	J-I	4.5×10^{-4}	9	− 1.13	− 1.19	− 1.17	− 1.15MPa
$\psi_\pi 100$	BCD1-KG1348D	6-6	J-I	6.4×10^{-4}	8.1	− 0.68	− 0.72	− 0.7	− 0.69MPa
Water-stress vs irrigated									
OA	BCD1-CDO497	6-6	J-I	2.9×10^{-4}	9	− 0.06	0.03	0.05	− 0.0006MPa

markers that had a significant effect on the trait variations found by interval mapping. However, the QTL found for ψ_π region D on chromosome 2 (2H) in the water-stress treatment and the QTL for $\psi_\pi 100$ in region H on chromosome 6 (6H) in the irrigated treatment were not identified. Two additional regions were detected in the water-stress treatment for ψ_π on chromosome 6 (6H) near the KG1348B locus ($P < 0.0014$) (region J) and for $\psi_\pi 100$ ($P < 0.003$) near the BCD266 locus (region E) on chromosome 2 (2H). At these loci, the Tadmor alleles resulted in higher values of the traits, explained 9% of the phenotypic variation, and were found on the total experiment and on the last five blocks respectively. The QTL for OA was found near the Dhn4 locus as previously observed by interval mapping, with the Tadmor alleles giving the more positive values, or lowering the capacity of OA. The ANOVA analysis was not applied for the four first blocks because of numerous missing data (too few RILs were represented in these blocks).

Some marker interactions were found to be significant at $P < 0.001$ (Table 5). The region A on chromosome 1 (7H), and especially the BCD1066 locus, acted in epistasis with two other loci in the water-stress treatment. For RWC, two QTLs previously identified interacted with the BCD1066–BCD1 loci. This interaction led to a $R^2 = 11\%$ of the phenotypic variation ($P < 0.00003$), and the combination of alleles of Tadmor and Er/Apm increased the RWC values. For $\psi_\pi 100$, the BCD1066-CDO1396A (this last marker was unlinked) interaction led to a $R^2 = 9\%$ of the phenotypic variance and the contribution of alleles of Tadmor and Er/Apm increased the $\psi_\pi 100$ values. The BCD1 locus acted in epistasis in the irrigated treatment for ψ_π, $\psi_\pi 100$, and in water-stressed vs irrigated treatment for OA: interactions were noted with a group of markers on another region of chromosome 6 (region H). In this case, the contribution of the two types of parental alleles decreased the osmotic-potential values.

Discussion

Several QTLs involved in OA variation

Despite strong environmental effects acting on the traits, several chromosomal regions related to variation in OA were mapped. As these traits are components, and are highly correlated with OA (Teulat et al. 1997b), the genomic regions found to have a control on both ψ_π and RWC or on $\psi_\pi 100$ could be considered to be involved in osmotic adjustment. The only QTL detected for OA was found on the same region of chromosome 6 (6H) (region J) as QTLs for several OA-related traits and confirmed the strength of these correlations. Two DNA regions where QTLs were detected for several OA-correlated traits were on chromosome 1 (7H), region A, and on chromosome 6 (6H), region J. Several additional regions were noted for individual traits on regions B, C, D, E, F, G and H (Fig. 3). This underlined the importance of mapping the QTLs involved in all the traits studied even though they are strongly correlated (Teulat et al. 1997a). However, we cannot exclude the possibility that other QTLs could have been detected using a more saturated map and with less environmental effects. The QTLs identified in this study must be therefore considered as a miminal number of the QTLs involved in OA variation.

Effects of the QTLs

All the DNA regions involved in OA and OA-correlated trait-variation showed additive genetic effects on the traits. Up to now, no data about epistatic effects in drought-tolerance studies have been published, but some examples of marker interactions increasing disease resistance were noted by Lefebvre and Palloix (1996) in pepper and by Besnard et al. (1997) in sunflower. In our study, some DNA regions (A, I and J) acted in epistasis (Table 5). In the water-stress treatment, the BCD1066-CDO1396A interaction decreased the $\psi_\pi 100$ values, showing a favorable influence of the susceptible parent genetic background for these markers. A similar effect has been demonstrated by Lefebvre and Palloix (1996) for *Phytophtora capsici* Leonian resistance in pepper. In the irrigated treatment, the BCD1 locus acted in epistasis with KG1348D for both ψ_π and $\psi_\pi 100$. These interactions decreased the ψ_π and $\psi_\pi 100$ values of the plants through a constitutive osmotic capacity. The plants had a low leaf osmotic potential at full turgor without a period of water stress. The region near Dhn4 also revealed positive additive effects for ψ_π, $\psi_\pi 100$, and OA, showing that the stressed ψ_π and $\psi_\pi 100$ values were higher than those obtained in the irrigated treatment and that the susceptible parent Er/Apm contributed to the expression of osmotic decrease in this cross. However, in the water-stress treatment some interactions between markers were favorable for increasing adaptive OA: the A region on chromosome 1 (7H) and the J region on chromosome 6 (6H) for RWC by additive and epistatic effects (increase of RWC) and BCD1-CDO497 interaction for OA (increase of OA). So, even if several QTLs involved in OA, and OA-correlated, trait-variation were identified on the same chromosomal region (J) we do not know if they contribute to the trait variation by a real OA or by constitutive osmotic capacity. As a consequence, adaptive and constitutive capacity are probably controlled by the same genomic regions and there might be different regulation mechanisms with respect to the water treatment. However, we cannot exclude the possibility that the results observed for the interactions when $P < 0.001$ were due to false positives. Interactions must be considered with caution.

Limits of QTL detection for plant water-status parameters

Environmental effects strongly influenced the water-status parameters and did not allow the detection of genetic variance for osmotic potential on the total experiment, whereas in the last five blocks the heritability of the trait could be evaluated and QTLs detected (Tables 2 and 3). In addition, the low heritability obtained for OA ($h^2 = 0.20$) could be explained by the fact that it was calculated as the difference between two traits which themselves were influenced by environmental effects. Consequently, the high residual variance was cumulated and influenced the power of the model estimating the components of variance. These environmental effects could also explain the low threshold values used for the detection of QTLs involved in water-relation parameters. Lebreton et al. (1995) detected putative QTLs involved in turgor and water potential on chromosome 3 in maize with a LOD > 2. The division of the results into two subgroups of plants that seemed to have been subjected to a different stress intensity has influenced the number of chromosomal regions identified as involved in the control of OA. However, these differences were not due to differences in the rate of soil drying as all the measurements were done at a soil moisture of 14% field capacity. Genetic variance for RWC explained the major genetic variation observed in the first four blocks, whereas ψ_π explained the genetic variation obtained for osmotic adjustment in the last five blocks which were less affected by the water stress. QTLs involved in ψ_π, $\psi_\pi 100$, and OA variation were only detected in these last blocks. These environmental differences could have been reduced if all the measurements had been done during the same experiment (only one block). But it would have also reduced the size of the population studied, which is important for QTL detection. Some genotype $\times$ environment interaction generated by accidental environmental effects may have influenced OA expression. This may be eventually assessed with multiple-environment QTL detection software (Tinker and Mather 1995).

A conserved region for OA in cereals

Morgan (1991) suggested a genomic location for osmoregulation on chromosome 7A in wheat by chromosome substitution, and Lilley et al. (1996) on the homoeologous portion of chromosome 8 of rice using a QTL approach. The comparison of the QTL positions detected in the present work with the regions previously suspected for OA variation revealed that a homoeologous region on chromosome 1 (7H) was found to be involved in OA in barley (region A). Lilley et al. (1996) have identified a QTL for OA at 70% of RWC on a small portion of chromosome 8 of rice (RG1-RZ66). The homoeologous region of this portion of the rice chromosome is flanked in barley by the BCD351A and CDO673 loci. In most barley consensus maps, the CDO595 marker co-segregates with the RZ66 marker and is located on this chromosomal segment close to the Acl3 locus. Morgan and Tan (1996) have recently located a putative gene for osmoregulation (*or*) on the short arm of chromosome 7A of wheat using RFLP analysis. This part of the chromosome is co-linear with the short arm of the barley chromosome 1 but *or* was located at a more distal position than in

barley and rice. The conserved genomic region found for OA in rice, in barley, and on the same co-linear chromosomal arm in wheat, should play a key role in drought adaptation as it was found using different experimental protocols and in plants at different stages of growth. Moreover several QTLs involved in root morphology and drought-avoidance-parameter variation in rice (Champoux et al. 1995), and the number of leaves under water stress in barley (Teulat et al. 1997a), were also identified on this chromosomal region. In the present work, several genomic regions for OA variation were detected, whereas in wheat Morgan (1991) concluded that OA has a monogenic control. Lilley et al. (1996) and Basnayake et al. (1995) suggested that more than one gene may control OA in rice and sorghum. The different results could be explained by the size of the RILs population. For example, in rice (Lilley et al. 1996) only 56 RILs were used. This low population size decreased the probability of QTL detection and could explain the fact that only one region with a major effect has been identified. When comparing our results with those obtained by Lilley et al. (1996) for lethal osmotic potential on a segment of chromosome 7 of rice, another chromosomal region was noted to be co-linear for ψ_π close to the E9_4 locus on chromosome 2 (2H).

Location of specific genes

The co-location of specific genes with QTLs could be a better way to understand the molecular basis of drought tolerance or of traits related to drought response. In this study, ψ_π and RWC QTLs co-segregated on chromosome 1 (7H) with the Acl3 locus (region A) coding for the barley acyl carrier protein III (Hansen and von Wettstein-Knowles 1991) whose role in drought tolerance has not been established. This gene encodes a co-factor protein of the plant fatty acid synthetase involved in the *de novo* synthesis of the fatty acyl chain, especially in chloroplasts. It could have a role in the protection of membranes or in membrane fluidity during stress. QTLs were also detected close to the Ss1B, Dhn4 and KG1348 loci, coding respectively for a sucrose synthase, a dehydrin and a thionin (Table 1). QTLs involved in several tolerance traits have already been identified near Dhn genes (Campbell and Close 1997) but the implication of these genes in tolerance remains to be proven. Quarrie et al. (1997) suspected the existence of a cluster of genes involved in stress tolerance.

Finally, the few studies on the location of QTLs involved in drought tolerance showed the difficulty in finding general key regions for adaptation. The dissection of each adaptive trait enables major chromosomal regions to be located. The validity of the regions was strengthened when related to other studies or to results obtained for different adaptive traits. The co-location of the QTLs detected for the different traits allowed us to identify two important genomic regions for OA and several other regions specific for one trait. Some of these could also be involved in OA but this remains to be proven. This illustrates the complexity of the genetic control of OA and emphasises that the perspective of marker-assisted selection (MAS) for OA will have to be carefully considered in barley. The several chromosomal regions involved, the important epistatic effects, and the role of the susceptible parent's genetic background, all underline this difficulty. But a chromosomal region seems to be conserved for OA variation among cereals. Therefore, if a genomic region would have to be transferred for OA improvement through MAS, this one would be a good target. Applying MAS using a QTL of major effect on OA and conserved among cereal species would allow rapid progress in introducing the trait into elite lines in a breeding program.

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