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New QTLs identified for plant water status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes

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Abstract Quantitative trait locus (QTL) analysis was carried out with 167 recombinant inbred lines (RILs) of barley derived from a cross between Tadmor and Er/Apm to identify the genomic regions controlling traits related to plant water status and osmotic adjustment (OA). The experiment was conducted in a growth chamber using a random incomplete block design (nine blocks). Relative water content (RWC) and leaf osmotic potential (ψ_π) were measured at 100% and 14% of the field capacity on 105 RILs in each block. In addition, the water-soluble carbohydrate concentration (WSC) was measured in the four first-blocks. The leaf osmotic potential at full turgor ($\psi_\pi 100$), the water-soluble carbohydrate concentration at full turgor (WSC100), and also OA, the accumulation of water-soluble carbohydrates (dWSC100), the contribution of a change in water content to OA (CWC) and of the net solute accumulation to OA (SA) have also been calculated. In a previous paper (Teulat et al. 1998), 12 QTLs were identified for RWC, ψ_π, $\psi_\pi 100$ and OA with adjusted means (block effects and pot-within-block effects fixed) with an incomplete genetic map. In the present paper, a more-saturated and improved map is described. A new QTL analysis as been performed with adjusted means. The new QTLs identified for previous evaluated traits, as well as the QTLs for the new traits, are presented. Eight additional regions (22 QTLs) were identified which increased to 13 the total number of chromosomal regions (32 QTLs) controlling traits related to plant water status and/or osmotic adjustment in this barley genetic background. The results emphasise the value of the experimental design employed for the evaluation of traits difficult to assess in genetic studies. The putative target regions for drought-tolerance improvement are discussed combining arguments on the consistency of QTLs and, when possible, the physiological value of QTLs (trait relevance, syntenic relationships and clustering of QTLs).

Keywords Mediterranean barley · Water stress · Water status · Osmotic adjustment · Quantitative trait loci

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Introduction

Drought is an important abiotic factor affecting the yield and yield stability of food cereals of the Mediterranean basin. This stress acts simultaneously on many traits leading to a decrease in yield. Drought tolerance could, therefore, be studied by identifying the traits which have a significant impact on yield, and the genetic factors controlling them. For this purpose, barley could serve as a simple genetic model as it is known to be well-adapted to several abiotic stresses, especially to water deficit (Ceccarelli 1987). The maintenance of relative water content (RWC) and a high osmotic adjustment (OA) are known to contribute to increase yield and yield stability under drought in cereals (Clarke and McCaig 1982; Morgan 1983; Schonfeld et al. 1988; Blum 1989; Matin et al. 1989). Osmotic adjustment is defined as a decrease of the osmotic potential within cells, due to an active solute accumulation after water-potential reduction in response to water stress (Blum 1988). For Wilson et al. (1980), OA could arise from an increase in the amount of solutes by active solute accumulation or a decrease in the water content on a dry weight basis. The decrease in osmotic potential leads to the maintenance of cell turgor, and more generally turgor-dependent processes, suggesting that OA is a good physiological trait to be considered in breeding for drought tolerance. The solutes which accumulate during OA include inorganic cations, organic acids, free amino acids and carbohydrates (Turner and Jones 1980). The main solutes accumulated during OA in barley are water-soluble carbohydrates (Lewicki 1993). According to Kameli and Lösel (1995), glucose could make the major contribution to OA in durum

wheat, whereas in barley sucrose seemed more important (Lewicki 1993). In addition, carbohydrates have been found to protect membranes and proteins against dehydration (Crowe et al. 1990).

Because the adaptative traits generally exhibit a continuous variation, a genetic approach through quantitative trait locus (QTL) detection, by using a genetic map, can be developed. This approach may improve understanding of the genetic basis of a trait (the number of loci controlling the trait variation, their effects, and favourable or unfavourable alleles). The major problem of the QTL strategy is that, in some cases, QTLs are specific to a given environment, growth stage, scoring method or genetic background. Consequently, the choice of the experimental design used in QTL studies of adaptative traits could also have an influence on the QTL consistency. In this context, an experimental design suitable for evaluating OA must be adapted to the constraints of a genetic and QTL study, and to the characteristics of the trait. The first requirement is addressed by testing a large number of genotypes with reproducible, rapid and numerous assessments. As OA implies a decrease of osmotic and water potentials and an active accumulation of solutes (Blum 1988), the experimental design must allow a distinction to be made between the concentration effects and the net solute accumulation. Measurements made at full turgor may allow this distinction, OA depending only on the amount of solute molecules. Osmotic potential measurement could therefore be performed after the re-hydration of a plant (Johnson et al. 1984; Blum 1989) or of an organ (Jones and Rawson 1979; Henson et al. 1982; Melkonian et al. 1982; Turner et al. 1987). However, during the re-hydration, uncontrolled metabolic effects could occur and interfere with OA. The osmotic potential without stress must also be considered, and a distinction made between an adaptative osmotic capacity which appears under stress and an intrinsic low osmotic potential without stress. For Ludlow et al. (1983), OA is defined as the difference between the osmotic potential at maximal turgor (Wilson et al. 1979) of the stressed and the unstressed plants. In this method, the evaluation of OA requires a comparison between well-watered plants and plants under a defined water stress. However, the definition of well-watered plants also differs according to authors (Jones and Rawson 1979; Basnayake et al. 1993). In order to manipulate the minimum of traits permitting the evaluation of OA for a genetic study, we have chosen to distinguish between the intrinsic and adaptative osmotic capacities, by measuring the traits at a given soil moisture for the water-stressed plants and on the same day for the corresponding irrigated plants. The calculation of the osmotic potential at maximal turgor proposed by Wilson et al. (1979) allowed the variation of osmotic potentials to be compared at a standardized RWC (100% RWC) at these soil moistures and the calculation of OA according to the method of Ludlow et al. (1983). The measurement of RWC was also a way to estimate the cell volume. A random incomplete-block design was chosen with nine blocks, each containing 105 recombinant inbred lines (RILs) per water treatment, as described in Teulat et al. (1998). In this previous paper we mentioned uncontrolled environmental variation (i.e. block and pot-within-block effects). However, the experimental design allowed us with a preliminary genetic map to identify the first QTL-controlling characteristics related to osmotic adjustment described in barley (RWC, leaf osmotic potential, leaf osmotic potential at full turgor and calculated OA), on chromosomes 7HS, 7HL, 2H, 1HL and 6HL using trait-adjusted means (block and pot-within-block effects fixed). A misunderstanding of this paper by Zhang et al. (1999) led to its strong criticism from a physiological point of view. These authors argued that the protocol used for identifying OA was not correct and that the plants were not stressed to a similar water status. However, the data were adjusted by the methodologies of Wilson et al. (1979) and Ludlow et al. (1983), which allow comparisons of plants at 100% of RWC, revealing their capacities to tolerate water stress. One QTL for calculated OA at 100% of RWC was mapped in this way on the long arm of chromosome 6H. In addition the adjusted means generated by fixing some significant environmental effects were used intentionally to limit environmental fluctuation. These adjusted means artificially restrict the range of RWC data compared to real phenotypic ones and cannot be taken as a physiological argument of an insufficient drought stress. The change in RWC in this experiment was unavoidable and, rather, represents a useful parameter for an evaluation of cell volume at a given soil-water status. RWC data allow both osmotic potential at maximal turgor and OA to be calculated (Wilson et al. 1979; Ludlow et al. 1983). Moreover, despite the concern of Zhang et al. that parental lines did not differ significantly for some traits, many geneticists also point out that QTL detection does not require a significant difference between the parental lines which may represent compensating allelic effects at different loci. Adjusted means were generated because non-significant differences between RILs for most traits were obtained using phenotypic data, as well as the high significant block and pot-within-block effects.

In the present study, in order to clarify the experimental design, avoid ambiguities and identify new consistent chromosomal regions controlling the variation of traits that have an impact on plant water status and osmotic adjustment, we re-visited the data with a more-saturated map and additional traits. The QTLs controlling RWC, leaf osmotic potential, contribution of a change in water content to OA, contribution of the net solute accumulation to OA and water-soluble carbohydrate concentration, as well as some traits calculated at full turgor (leaf osmotic potential, water-soluble carbohydrate concentration, accumulation of water-soluble carbohydrates and OA) were examined from the adjusted means. The consistency of the chromosomal regions found was discussed, in an attempt to combine physiological and genetical information in terms of water-deficit response or drought tolerance in cereals.

Table 1 Random incomplete block design used for the evaluation of osmotic adjustment in the aim of a QTL study. Each of the nine blocks contained 105 recombinant inbred lines (RILs) in each water treatment (14% and 100% FC), 21 pots per treatment and five RILs per pot . At the end of the experiment, 187 RILs and two parents (Tadmor, Er/Apm) were represented five times

RIL/block	1	2	3	4	5	6	7	8	9
1–21	x					x	x	x	x
22–42					x	x	x	x	x
43–63				x	x	x	x	x	
64–84			x	x	x	x	x		
85–105		x	x	x	x	x			
106–126	x	x	x	x	x				
127–147	x	x	x	x					x
148–168	x	x	x					x	x
169–189	x	x					x	x	x

Materials and methods

A population of 187 barley recombinant inbred lines (RILs) and the two parents of this progeny (Tadmor and Er/Apm) were studied under controlled conditions at an early stage of growth at the Genetic and Plant Breeding Laboratory of ENSA-INRA-Montpellier (France). Tadmor is a two-row variety selected from a Syrian landrace. It is well-adapted to the dry conditions (250–400 mm rainfall) of the Middle-East and characterised by high yield stability. Er/Apm is an improved two-row variety developed by ICARDA. It is adapted to moderate rainfall conditions. RILs were advanced by bulk until the F4 generation at ICARDA (Syria) and then by single-seed descent until the F8 generation at CIMMYT (Mexico).

The details of the experimental design and conditions were described in Teulat et al. (1998). To summarise, a random incomplete-block design containing nine blocks was performed (Table 1). To allow the calculation of OA according to the methodology of Ludlow et al. (1983), two sets of 105 plants were grown in each block under two water regimes: one set under water deficit and the other set with an irrigation supply (21 pots with five different RILs per pot for each water treatment). The water stress was imposed at the 4-leaf stage by stopping the irrigation for the first set of plants while the other set was maintained well-watered. After 12 days, the relative soil moisture content was 14% of the field capacity (FC) for the stressed-plants and 100% FC for the irrigated plants (pots were weighed and watered daily). At the end of the experiment, five replicates of each of the 187 RILs were evaluated per water treatment (Table 1).

The measurements focused on water status and OA parameters. Leaf RWC was evaluated according to Barrs and Weatherley (1962): RWC(%) = (FW-DW) × 100 / (TW-DW) where FW was the fresh weight, DW the dry weight and TW the turgid weight. It was measured on the last fully expanded leaf to avoid possible interaction between OA and growth (Munns 1988; Li et al. 1993) on samples collected after 6 h of light. Then, the penultimate leaf was wrapped in aluminium foil, frozen in liquid nitrogen and stored at –20°C. The leaf osmotic potential (ψ_π) was measured on these samples using a freezing-point micro-osmometer (Roebling 13 GS/IS). Osmotic potential at full turgor ($\psi_\pi 100$) was calculated according to Wilson et al. (1979) as $\psi_\pi 100 = \psi_\pi \times (RWC\text{-}B) / (100\text{-}B)$, where B is the apoplastic water content. The barley B value of 5.8% was used (Tetlow and Farrar 1993). The water-soluble carbohydrate concentration (WSC) was also determined at the two soil-moisture contents according to Dubois et al. (1956) from 100 mg of fresh leaf, but only on the 4-first blocks. The WSC concentration at full turgor (WSC100) was calculated in addition as WSC100 = WSC × (RWC-B) / (100-B).

Osmotic adjustment was then calculated according to Ludlow et al. (1983) as $OA = \psi_\pi^c 100 - \psi_\pi^s 100$, where $\psi_\pi^s 100$ is the $\psi_\pi 100$ of the stressed sample at 14% FC and $\psi_\pi^c 100$ is the $\psi_\pi 100$ of the well-watered control sample at 100% FC. Osmotic adjustment represents therefore the difference between the two $\psi_\pi 100$ values estimated in irrigated and stressed conditions at a given soil moisture (Flower and Ludlow 1986). As for OA, and to allow a comparison with OA, the accumulation of WSC was calculated at full turgor by the difference between the WSC100 from the stressed and the irrigated plants as $dWSC100 = WSC100^s - WSC100^c$, where WSC100 = water-soluble carbohydrate concentration at full turgor, c = well-watered sample and s = stressed sample. In addition, as OA could come both from an increase of solutes or a decrease in the water content, the contribution to OA of a change in the water content (CWC) was calculated according to Ludlow et al. (1983) as $CWC = (\psi_\pi^c 100 \times ((TW / DW)_c\text{-}1 / (TW / DW)_s - 1) - \psi_\pi^c 100)$, where TW is the leaf turgid weight, DW the leaf dry weight, s the water-stressed plants and c the control irrigated plants. Then, the contribution of the net solute accumulation (SA) to OA was calculated by the difference between OA and CWC (Ludlow et al. 1983).

For the detection of marker-trait associations, phenotypes of a subset of 167 RILs have been analysed with 118 molecular markers. A preliminary map was described in Teulat et al. (1998). To fill the gaps, 15 RFLP, four RAPD, one simple sequence repeat (SSR) and 77 amplified fragment length polymorphism (AFLP) markers were added to this basic map using the same procedure as in Teulat et al. (1998) for RFLPs and RAPDs. For the AFLP generation, DNA restrictions and ligations were performed as described in the GibcoBrl AFLP Analysis System I instruction Manual. A sample of 0.25 µg of barley DNA was restricted with *Eco*R1 and *Mse*1. The digestion products were ligated to *Eco*R1 and *MseI* adapters. Pre-amplifications were performed with primers each having one selective nucleotide. Then, the PCR products were 1/10 diluted and 5 µl of DNA was used for the final selective amplification. Seven primer combinations were tested: M-CAA/E-AAG, M-CTC/E-AAG, M-CTG/E-ACC, M-CTA/E-ACC, M-CAA/E-ACC, M-CTT/E-ACC and M-CTA/E-AAG. Out of a total of 209 markers available (one SSR, 36 RAPDs, 77 AFLPs, two morphological markers, 93 RFLPs), only 118 (38 AFLPs, one SSR, one morphological marker, two RAPDs and 76 RFLPs) were used for the map construction. The other markers were discarded when the data were recorded for less than 90 RILs, when the strength of the order of the markers on the chromosomes was weak, when the markers were co-located or when the markers were distorted (generally dominant ones) at $P < 0.01$. The new map was constructed with MAPMAKER version 3.0b (Lander et al. 1987) using the Kosambi mapping function (Kosambi 1944) (a minimum LOD score of 3 and d_{max} = 37.2 cM). The 76 RFLP markers constituting the basic map originated from 70 clones from diverse sources including genomic DNA and cDNAs: one clone from rice (rz), 20 from oat (CDO), 21 from barley (12 BCD, five MWG and one cMWG, two ABG and one ABC), 16 from wheat (WG), one from corn (BNL), one from sorghum (SB) and ten clones representing candidate genes (references in Teulat et al. 1998). Sixty four clones have revealed one locus, five have revealed two loci and one five loci, of which three were discarded. A locus for ribulose biphosphate carboxylase activase (*rbcac*) was added to the first map by amplification using specific primers of a simple sequence repeat within the gene (Rundle and Zielinski 1991).

Adjusted means (means generated by fixing the block and the pot-within-block effects, Teulat et al. 1998) were analysed by interval mapping using MAPMAKER/QTL version 1 (Lander and Botstein 1989) and single-factor analysis of variance with QGene version 2.30 (Nelson 1997) for distal regions and markers flanking major gaps. A LOD score ≥ 2.0 and $P < 0.005$ was used to declare the putative QTL as significant. As well, as in Teulat et al. (1998), the means from two more homogeneous subsets (4-first blocks and 5-last blocks) and from the total experiment (all nine blocks) were considered.

Results

Advances in the barley genetic map

The markers added to the preliminary genetic map (RFLPs, AFLPs and SSRs) have considerably improved

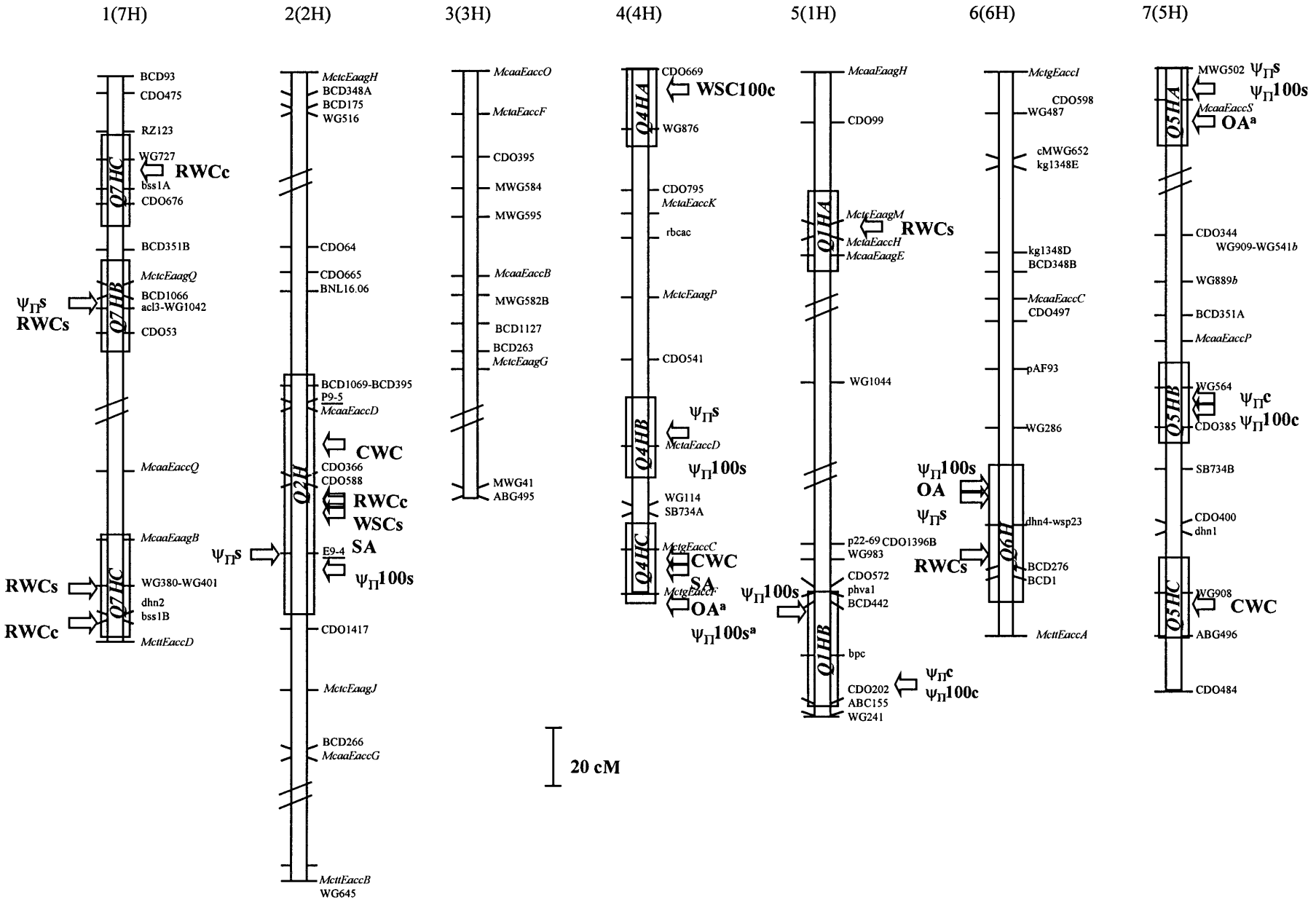
1(7H)
2(2H)
3(3H)
4(4H)
5(1H)
6(6H)
7(5H)
BCD93
CDO475
RZ123
WG727
RWCc
bss1A
CDO676
Q7HC
BCD351B
MctcEaagQ
BCD1066
acl3-WG1042
ΨΠs
RWCs
CDO53
Q7HB
McaaEaccQ
McaaEaagB
RWCs
WG380-WG401
dhn2
bss1B
RWCc
Q7HC
McttEaccD
MctcEaagH
BCD348A
BCD175
WG516
CDO64
CDO665
BNL16.06
BCD1069-BCD395
P9-5
McaaEaccD
CWC
CDO366
CDO588
RWCc
WSCs
SA
ΨΠs
E9-4
ΨΠ100s
Q2H
CDO1417
MctcEaagJ
BCD266
McaaEaccG
McttEaccB
WG645
McaaEaccO
MctaEaccF
CDO395
MWG584
MWG595
McaaEaccB
MWG582B
BCD1127
BCD263
MctcEaagG
MWG41
ABG495
CDO669
WSC100c
Q4HA
WG876
CDO795
MctaEaccK
rbcac
MctcEaagP
CDO541
ΨΠs
Q4HB
MctaEaccD
ΨΠ100s
WG114
SB734A
MctgEaccC
CWC
SA
Q4HC
MctgEaccF
OAa
ΨΠ100sa
McaaEaagH
CDO99
MctcEaagM
RWCs
Q1HA
MctaEaccH
McaaEaagE
McaaEaccC
CDO497
pAF93
WG1044
WG286
ΨΠ100s
OA
ΨΠs
dhn4-wsp23
Q6H
RWCs
BCD276
BCD1
MctgEaccI
CDO598
WG487
cMWG652
kg1348E
kg1348D
BCD348B
MctaEaccA
p22-69
CDO1396B
WG983
CDO572
phva1
BCD442
ΨΠ100s
bpc
Q1HB
ΨΠc
ΨΠ100c
CDO202
ABC155
WG241
MWG502
ΨΠs
ΨΠ100s
McaaEaccS
OAa
Q5HA
CDO344
WG909-WG541b
WG889b
BCD351A
McaaEaccP
WG564
ΨΠc
Q5HB
CDO385
ΨΠ100c
SB734B
CDO400
dhn1
WG908
CWC
Q5HC
ABG496
CDO484
20 cM

Table 2 Phenotypic means, standard deviation (SD) and ranges of variation (minimum / maximum values) of the 187 recombinant inbred lines (RILs) and the two parental genotypes for the different traits within the two water treatments, water-stress and irrigated, and for traits calculated from traits measured at the two field capacities. The RIL, block and pot (within block) effects obtained from an analysis of variance with these parameters as factors are also indicated. RWC: relative water content, ψ_π: leaf osmotic potential, ψ_π100: leaf osmotic potential at full turgor, WSC: water-soluble carbohydrates, WSC100: WSC at full turgor, FW: fresh weight, OA: osmotic adjustment calculated at 100% RWC, SA: net solute accumulation contributing to OA, CWC: contribution to a change in water content to OA (Ludlow et al. 1983), and dWSC100: accumulation of water-soluble carbohydrates

Item	Tadmor		Er/Apm		RILs		Range	RIL	Block	Pot(block)
	Mean	SD	Mean	SD	Mean	SD				
Water stress										
RWC (%)	94.13	3.93	89.47	9.03	82.34	16.29	35.44 / 99.93	0.022*	0.0001***	0.0001***
ψ_π (MPa)	–1.14	0.09	–1.31	0.29	–1.61	0.69	–4.40 / -0.36	0.418 ns	0.0001***	0.0001***
ψ_π100 (MPa)	–1.03	0.04	–1.14	0.17	–1.20	0.22	–2.54 / -0.32	0.047*	0.0001***	0.1440 ns
WSC (mg gFW^{-1})[a]	–	–	–	–	9.51	1.77	1.14 / 15.04	0.183 ns	0.457 ns	0.454 ns
WSC100[a]	–	–	–	–	6.45	1.87	0.61 / 12.55	0.882 ns	0.0001***	0.0001***
Irrigated										
RWC	99.26	1.29	98.52	1.95	98.34	2.27	95.51 / 100.00	0.626 ns	0.0001***	0.0002***
ψ_π	–1.04	0.02	–1.18	0.04	–1.15	0.14	–2.42 / -0.84	0.328 ns	0.0001***	0.0103*
ψ_π100	–1.03	0.03	–1.16	0.03	–1.13	0.13	–2.41 / -0.82	0.325 ns	0.0001***	0.0041***
WSC[a]	–	–	–	–	2.33	0.55	1.4 / 4.28	0.911 ns	0.032*	0.910 ns
WSC100[a]	–	–	–	–	2.25	0.54	1.03 / 4.15	0.862 ns	0.016*	0.851 ns
Traits calculated from the two treatments										
OA[b] **(MPa)**	–	–	–0.014	0.15	0.073	0.23	–0.80 / 1.52	0.275 ns	0.0001***	0.725 ns
SA[b] **(MPa)**	–	–	0.34	0.89	0.194	0.39	–0.85 / 2.23	0.417 ns	0.0001***	0.537 ns
CWC (MPa)	–0.439	0.48	–0.356	0.87	–0.122	0.27	–1.91/1.14	0.501 ns	0.0001***	0.0001***
dWSC100[a]	–	–	–	–	7.25	1.89	–1.38 / 13.72	0.235 ns	0.156 ns	0.458 ns

*, **, *** indicate differences significant at $P < 0.05$, 0.01 and 0.001 respectively or not significant (ns)

[a] Measured only on the 4-first blocks, parental values not available
[b] Only one value for Tadmor

the genome coverage (Fig. 1). We obtained 15 linkage groups assigned to the seven barley chromosomes. Without considering the main gaps, the new map represents a minimum of 1,101 cM. The previous unassigned group 8 (Teulat et al. 1998) was mapped on chromosome 1H. Important improvements of chromosomes 4H, 1H and 5H were obtained and the *rbcac* gene was mapped on chromosome 4H, as in the literature (Becker and Heun 1995). The main gaps remained near the centromeres for chromosomes 7H and 1H, on the short arms of chromosomes 2H and 5H and on the long arm of chromosome 3H. When comparing with other published barley maps, these gaps are not important in term of genetic distance; the biggest one corresponding to the centromeric region of chromosome 7H.

◀ **Fig 1** Genetic barley map based on the RIL population from the cross between Tadmor and Er/Apm. The map distances are expressed in cM (Kosambi function) and the markers indicated on the right of the chromosome skeleton: AFLPs in *italics* and RAPDs *underlined*. The short arms are represented at the top. The *inclined lines* represent the main gap locations. *b* Distorted marker at a 1% level. *Arrows* indicate the maximum likelihood for QTLs identified from interval mapping analysis for osmotic potential (ψ_π), ψ_π at full turgor (ψ_π100), water-soluble carbohydrate concentration (*WSC*), WSC at full turgor (*WSC100*), relative water content (*RWC*), osmotic adjustment (*OA*), contribution of a change in water content to OA (*CWC*), and contribution to a net solute accumulation to OA (*SA*) from the adjusted means. The QTLs on the right are the new QTLs identified in the present paper and those on the left are those previously identified in Teulat et al. (1998). The chromosomal regions where QTLs were detected are also indicated and named Q7HA to Q5HC in italics on the right of the chromosomes. The name of the traits is followed by *c* control irrigated treatment (100% FC), or *s* water stress treatment (14% FC). *a* additional QTLs from single-factor analysis of variance

Phenotypic variation of the traits

The adjusted means of RWC, ψ_π, ψ_π100 and OA were initially presented in Tables 2 and 3 of Teulat et al. (1998). Here, the phenotypic data for all the traits are reported in Table 2 for the total experiment. A large variation was observed in the progeny from the phenotypic data for all the traits in the two water treatments. In general, the range of variation was higher for the water-stressed RILs compared to the irrigated RILs. For example, ψ_π varied between –4.40 MPa and –0.36 MPa and the RWC from 35.44% to 99.93% at 14% FC, whereas the same traits varied respectively between –2.42 MPa to –0.84 MPa and 95.51% to 100% at 100% FC. In addition, highly significant differences were obtained between the two water treatments for all the traits ($P < 0.0001$). The values obtained at 100% FC were significantly higher for RWC, ψ_π and ψ_π100, and lower for WSC and WSC100 compared to the water-stressed values ($P < 0.0001$). As an example, the concentrations of WSC were 9.51 mg per gFW at 14% FC and 2.33 mg per gFW at 100% FC. At 14% FC, the values of WSC100 were lower than those of WSC. However, they were still higher (6.45 mg per gFW) than the WSC100 values obtained at 100% FC

Table 3 Additional QTLs detected by interval mapping for relative water content (RWC), osmotic potential (ψ_π), ψ_π at full turgor (ψ_π100), water-soluble carbohydrate concentration (WSC) for the water-stressed and the irrigated RILs, for osmotic adjustment (OA100% RWC), contribution to a change in water content to OA (CWC) and net solute accumulation contributing to OA (SA) from adjusted means of the total experiment; [4]the first-4 blocks and [5]the last-5 blocks. %Var: individual variance explained by the QTL. a: additional QTLs from a single-factor analysis of variance at telomeric markers or markers flanking major gaps. *P*: probability of significance. R^2: part of the explained variation

Traits	*Chromosomal region*[a]	*Left marker (distance to peak LOD in cM)*	*LOD*	*Estimated additive effects*[b]	*%Var*
Water stress					
RWC	Q1HA	MctcEaagM[5] (2)	2.10	1.26	12.3
ψ_π	Q4HB	CDO541 (30) and [5] (MctaEaccD+0)	2.44 (2.68)	–0.08 (-0.07)	7.9 (13.1)
	Q5HA	MWG502[5] (8)	3.58	0.09	20.0
ψ_π100	Q2H	E9–4[5] (14)	2.26	0.06	17.1
	Q4HB	MctaEaccD[5] (0)	2.21	–0.05	10.9
	Q4HC	MctgEaccF[4a]	$P < 0.0018$	–	***14.2***
	Q5HA	MWG502[5] (9)	2.62	0.05	13.3
WSC	Q2H	CDO588[4] (18)	3.02	–108.58	18.6
Irrigated					
RWC	Q7HA	WG727[4] (6)	2.24	1.21	12.2
	Q2H	CDO588[4] (8)	2.49	1.27	13.6
ψ_π	Q1HB	CDO202[5] (0)	2.33	0.06	11.4
	Q5HB	WG564[5] (6)	2.13	0.06	13.0
ψ_π100	Q1HB	CDO202[5] (0)	2.33	0.06	11.3
	Q5HB	WG564[5] (6)	2.15	0.07	13.9
WSC100	Q4HA	CDO669[4] (2)	2.45	20.50	10.9
Traits with two treatment components					
OA100%RWC	Q4HC	MctgEaccF[4a]	$P < 0.0013$	–	***15.6***
	Q5HA	McaaEaccS [a] and [4a]	$P < 0.0039$ (0.0043)	–	*5.0 (10.77)*
CWC	Q2H	McaaEaccD (0) and [4] (p9–5+0)	3.57 (5.6)	–0.119 (-0.267)	11.9 (20.6)
	Q4HC	MctgEaccC (6) and [4] (MctgEaccC+8)	3.11 (2.24)	–0.121 (-0.215)	12.4 (12.9)
SA	Q5HC	WG908 (0) and [4] (dhn1+20)	2.34 (2.92)	–0.088 (-0.223)	6.5 (15.0)
	Q2H	CDO588 (0) and [4]	2.45 (2.75)	0.11 (0.23)	6.6 (10.7)
	Q4HC	MctgEaccC (6)	3.38	0.17	14.1

[a] Refer to Fig. 1
[b] The estimated genetic effect is expressed in the trait unit, contribution of Tadmor's alleles compare to Er/Apm

(2.25 mg per gFW). The phenotypic mean value for OA was low (0.07 MPa) but some RILs presented high OA capacity, the maximum value being 1.52 MPa. Conversely, some RILs, as well as Er/Apm, presented a lower ψ_π100 value in the irrigated treatment compared to the water-stressed treatment, leading to negative OA values. The phenotypic mean values of the contributions of SA and CWC to OA were 0.19 MPa and –0.12 MPa respectively. Finally, the mean phenotypic value of dWSC100 was high (7.25 mg per gFW) with a maximum of 13.57 mg per gFW.

A significant RIL effect was only detected for RWC and ψ_π100 at 14% FC. For most of the traits the block and pot-within-block effects were highly significant which could explain why the RIL effects were not significant. Different RIL effect values were obtained for the two subsets of means of the 4-first and 5-last blocks (data not shown). For example, significant RIL differences were obtained at 100%FC for ψ_π and ψ_π100 with data of the 5-last blocks, and for SA and CWC in the 4-first blocks. But in all sets of means, and for all the traits, the adjustment of the data by fixing the block and the pot within-block effects in this experimental design allowed part of these environmental effects to be reduced (Teulat et al. 1998). With the adjusted means, a large trait variation was also observed and an important water-treatment effect was obtained for all the traits ($P < 0.0001$).

Identification of new QTLs

Table 3 describes the genetic characteristics of the new QTLs detected in chromosomal regions not previously mapped in Teulat et al. (1998) for RWC, ψ_π, ψ_π100 and OA, and considering all the maps for the new traits WSC, WSC_{100}, CWC and SA. Figure 1 summarises the location along the seven barley chromosome map of the new QTLs as well as those previously identified in the first paper. The chromosomal regions where significant marker-trait associations have been identified have been given the prefix Q, followed by the name of the chromosome and one letter referred to their chromosomal position (A to C, depending on the number of regions).

With the more-saturated and relevant genetic map, ten QTLs among 12 previously identified with adjusted means in Teulat et al. (1998) for RWC, ψ_π, ψ_π100 and OA were confirmed. With the improvement of some map regions, two QTLs became non-significant: one for ψ_π

on chromosome 2H near WG645 and MWG720 (water-stressed RILs), and one for $\psi_\pi 100$ on chromosome 6H near Kg1348 E (irrigated RILs).

The new QTLs mapped at 14% FC for RWC were in Q1HA, for ψ_π, in Q4HB and Q5HA, and for $\psi_\pi 100$ in Q2H, Q4HB, Q4HC and Q5HA. The LOD scores varied from 2.10 to 3.58, and the part of explained variation from 7.9 to 20%. Only one QTL was identified for WSC variation under water stress (Q2H) (Table 3 and Fig. 1).

At 100% FC, two new regions were associated with RWC (Q7HA and Q2H), two with ψ_π (Q1HB and Q5HB), and two with $\psi_\pi 100$ (Q1HB and Q5HB). No significant QTL was found from the irrigated RILs for WSC but a QTL was detected for WSC100 in Q4HA. For this water treatment, the LOD scores varied from 2.13 to 2.49 and the part of explained variation from 10.9 to 13.9%.

Concerning OA, in addition to the QTL previously identified from the 5-last blocks in Q6H (Teulat et al. 1998), significant QTLs were detected by single-factor analysis of variance at the distal marker in Q4HC, and at the marker flanking the gap in Q5HA. QTLs controlling CWC were identified in Q2H, Q4HC and Q5HC and those controlling SA were in Q2H and Q4HC (Table 3; Fig. 1).

Discussion

Eight new chromosomal regions (22 QTLs) were identified (Table 3) leading to a total of 13 QTL regions (32 QTLs) (Table 3; Fig. 1 and Teulat et al. 1998) controlling traits related to plant water status and/or osmotic adjustment in the barley genetic background studied. It is necessary to identify the most-consistent and important of these QTLs, in term of improving drought tolerance, based on the whole analysis. Were some QTLs associated with drought tolerance? Were others only involved in a dehydration response, or in both of these characteristics? The resolution of these questions depends on the statistical consistency of the QTLs as well as on their physiological impact.

Consistency of QTLs emerging from a random incomplete-block design

To analyse a great number of RILs and a minimum number of plants per RIL, a balanced random incomplete-block design was chosen. This design also allowed us to take into account an eventual heterogeneity that could exist between blocks. To limit this possible heterogeneity, the experiment was conducted under controlled conditions. As previously mentioned in Teulat et al. (1998), each block was performed chronologically between October 1994 and February 1996 because of the small area of the growth chamber and the number of trait measurements that needed to be carried out simultaneously. It would not have been possible to do all the measurements and all the samplings on more than 1,600 plants simultaneously, and to monitor plant water status daily for 378 pots at 14% FC and at 100% FC by doing the experiment in a greenhouse at one time. However, our results showed that, even under controlled conditions, testing a great number of genotypes is difficult without excluding environmental effects and experimental limitations. For this reason, the value of the design is reinforced as it allowed us to obtain QTLs from the adjusted means generated from two subsets of more homogeneous blocks (first-4 blocks and last-5 blocks) and from all blocks. Each of these data sets contained a sufficient number of individuals to perform a correct QTL analysis: 85 RILs for the first-4 blocks, 105 RILs for the last-5 blocks and 167 RILs for all the 9-blocks with a minimum of three plants per RIL. For the whole experiment, five plants from each RIL were used to maximise precision. This number was higher than that used in other studies on OA (Lilley et al. 1996) and may explain why fewer QTLs were obtained by these authors. When performing the analysis directly with the phenotypic data obtained by means across blocks, few QTLs would have been obtained because, for most traits, no differences between RILs were found and the environmental effects would have certainly hidden the genetic variation. However, with the adjusted means, the LOD scores for QTL detection were still low, even if statistically significant. These QTLs could have been more consistent in other or more-controlled experimental conditions. In addition, one should not forget the limitations of any statistical data obtained from the calculated parameters, and the QTLs identified for such traits should be considered as first indicators that must be strengthened by other data. For example, in Teulat et al. (1998) a portion of chromosome 7H (Q7HB) was pointed out as a potential region of interest for OA control not only from QTL data but also from syntenic information.

Physiological value of QTLs

In a genetic study the traits are measured under standardized and often simplified conditions (e.g. given soil moisture, growth stage), and from this point of view it is difficult to give a physiological meaning to a QTL (This and Teulat-Merah 1999). However, to be relevant to plant improvement, the traits employed and the QTLs identified must be assessed according to their physiological effect on reducing yield losses under drought. Preliminary information can be obtained from a genetic evaluation: they could come: (1) from a correlative approach (correlation between traits) conducted on a large population, (2) from a comparison of results obtained at several soil water status levels (here 14%FC and 100%FC) or standardised at 100% RWC by calculation, and finally (3) from a co-location (QTL-QTL, candidate genes-QTL) analysis within the same species or with related species taking into account syntenic relationships.

Nine of the thirteen chromosomal regions with QTLs identified in this study concerned more than one trait (Fig. 1). In Q7HB, two QTLs were co-located: one for

RWC and one for ψ_π. Both traits gave an idea of the plant water status at 14%FC. The co-location on chromosome 2H of QTLs for WSC, several plant water-status traits, CWC and particularly SA, was also noticed. Traits such as WSC and $\psi_\pi 100$ were not *a priori* related, but the existence of common QTLs in the region have underlined the possible link between them under water stress. It was the same for WSC and SA associated with a QTL in the same area, suggesting a possible contribution of water-soluble carbohydrates in the solutes accumulated during OA in our cross. However, even if this hypothesis is in accordance with previous results obtained from the parental genotypes (Teulat et al. 1997b), and from observations made by Lewicki (1993) suggesting that WSC were the solutes mostly accumulated during OA in barley, the role of this QTL in controlling solute content contributing to OA remains to be proven. A highly significant and positive correlation found between the accumulation of WSC (dWSC100) and RWC (r = 0.73***) under water stress also suggested that the more RILs that were able to maintain their RWC, the more they have accumulated WSC and vice versa. This underlined the fact that, globally in the cross, an important part of WSC under stress came from a real increase of the number of molecules rather than from a dehydration of the leaf tissues.

The QTL co-location analysis could shed some light on the basis of the genetic and phenotypic correlations. For example, when co-locations were extended to other traits measured on the same genetic background and in the same experiment, the presence of a QTL for a growth parameter in the Q7HB and Q7HC areas suggested a physiological link between water status and growth regulation (Teulat et al. 1997a). As another example, six of the seven QTLs detected for chlorophyll content were mapped in areas identified for traits presented in the present paper (Q7HB, Q7HC, Q2H, Q4HA, Q4HC and Q5HC) (This et al. 2000). The relationship between carbon metabolism and early growth in maize was strengthened by the common locations of QTLs for the different traits (Causse et al. 1995). In all these cases, the co-locations suggested a common genetic control of the traits that could lead to causal relationships.

The strategy employed has revealed which regions were specific to a given water treatment or which ones were revealed in both treatments (Q7HB, Q4HB, Q4HC, Q1HA, Q6H and Q5HA at 14%FC; Q7HA, Q4HA and Q5HB at 100%FC; and Q7HC, Q2H or Q1HB at both FCs). The same was obtained in maize where four QTLs related to invertase activity and hexose content were identified as either under control or under water stress conditions, other QTLs being effective under one of the latter conditions (Pelleschi et al. 1999). In the present study, several regions were also specific to the water stress response, which could be of interest for marker-assisted selection when favourable alleles are selected. The traits calculated at full turgor, such as $\psi_\pi 100$ and WSC100, are interesting to study under water stress, and favourable alleles at their QTLs could be selected and accumulated. They allow one to compare the variation within the RILs of ψ_π and WSC at a given water status level, 100%RWC. The alleles leading to a lower $\psi_\pi 100$ and a higher WSC100 under stress could contribute to a lower dehydration effect, helping drought tolerance. This is also true for RWC in the water-stressed treatment. The trait was considered interesting when the allele effect at a QTL was in favour of a higher RWC under stress; the maintenance of RWC, together with a high OA capacity, being in favour of turgor maintenance and contributing to yield stability under drought conditions in cereals (Clarke and McCaig 1982; Morgan 1983; Blum 1988; Schonfeld et al. 1988; Matin et al. 1989). The intrinsic ability to accumulate solutes also has a physiological significance for drought tolerance. This capacity was detected for the susceptible parental genotype Er/Apm and for some of the RILs in our cross (Teulat et al. 1997b, 1998). Therefore, QTLs for $\psi_\pi 100$ and WSC100 identified from the irrigated RILs could also be considered as leading to a passive or constitutive adaptation. However, the traits of main interest remain OA, dWSC100, CWC and SA, as they take into account parameters from the water-stressed, as well as the irrigated, treatment (the intrinsic ability under optimal conditions).

The comparison of QTL positions controlling the same traits in other populations or species via the syntenic comparison is a way to test the consistency of the QTLs over genetic backgrounds or environments. These types of comparisons have been made between three rice populations and have underlined the most consistent QTLs for root growth (Price and Tomos 1997). In Teulat et al. (1998), the Q7HB region was emphasised because it controlled the variation of RWC and ψ_π at 14%FC in barley and is common to the major QTL found by Lilley et al. (1996) for OA 70%RWC in the homoeologous portion of rice chromosome 8 (Teulat et al. 1998; This and Teulat-Merah 1999). This suggested that the barley Q7HB region could also be involved in OA control. This hypothesis was reinforced by the presence in the region of a non-significant QTL for OA at a LOD score of 1.8. The presence of a QTL for RWC in this area is not a reason to eliminate its possibility to control OA, the two traits being highly correlated (Teulat et al. 1997a). Zhang et al. (1999) presented a figure where the gene *or* (Morgan and Tan 1996) that could be involved in OA in wheat, seemed to be colinear to Lilley's QTL for OA. This, however, is not true because the gene is mapped in a region of chromosome 7 A distant from a minimum of 50 cM to the portion corresponding to rice chromosome 8. The gene *or* is linked to the xpsr119 marker and the region could correspond to a portion of rice chromosome 6. Indeed, the small arm of *Triticeae* chromosome group 7 could correspond at least to rice chromosomes 6 and 8. A more precise comparative mapping of this region may solve these ambiguities.

When the comparisons were extended, a QTL found for lethal ψ_π variation on rice chromosome 3 (Lilley et al. 1996) was mapped in the homoeologous portion of

barley Q5HC, where a QTL for CWC has been detected (Fig. 1). The lethal ψ_π is a trait related to dehydration and could be considered as an indicator of dehydration resistance. Elucidating the molecular basis of this variation in both species may provide new interest for this region in barley even through a statistical limitation of its value. The region Q2H is also interesting for several traits (ψ_π, ψ_π100, WSC, SA) in barley. A QTL for lethal ψ_π variation on rice chromosome 7 (Lilley et al. 1996) could correspond to this barley homoeologous region near CDO588 (Teulat et al. 1998), and a QTL for the sum of fructose and glucose on a portion of chromosome 10 of maize near CDO1417 (Pelleschi et al. 1999). Finally two QTLs were identified by Lilley et al. (1996) in rice for RWC at –3.5 MPa, a trait evaluating OA: one on chromosome 8 (syntenic with Q7HB) and the other on chromosome 5 (syntenic with Q1HA where a QTL for RWC was identified for the water-stressed conditions). None of the regions found in the present study as involved in OA directly (Q4HC, 6H and Q5HA) or suggested by syntenic relationships (Q7HB and Q1HA) are homologous to rice regions involved in dehydration tolerance or resistance via lethal ψ_π in rice. The study of QTL synteny is then an interesting tool to compare the validity of QTLs and to allow comparative physiology in a group of related species.

Conclusion

The QTL analysis presented in this paper has shown the value of the experimental design employed, even with experimental difficulties, and the interest of producing adjusted means. They have allowed the detection of the most-consistent QTLs resulting from nine experiments, eliminating part of the environmental effects. QTLs were identified for criteria known to contribute to drought tolerance or to traits with a physiological impact on drought tolerance: RWC, OA, CWC, SA and dWSC100. There was sometimes no clear evidence whether the regions identified reflected only a water stress response, the variation coming more from tissue dehydration, or an adaptative response, the variation coming from an active increase of solutes. The great number of regions identified under water stress underlines the difficulty to produce a simple scheme for marker-assisted selection (MAS) and the interest of comparing results with similar work performed on related species, such as rice, wheat or maize, in order to help in the selection of the most-consistent targets. Progress in comparative mapping and micro-synteny analyses are however required before any QTL comparison can be made without any doubt. Several authors proposed that a strategy based on the introduction of several advantageous traits could improve yield under drought but, realistically, how many traits should be followed and how many regions identified? In the studied genetic background, the ''susceptible'' parent Er/Apm also presented favourable alleles at QTLs, which suggests that both parental lines may provide favourable alleles. Finally, the link with field performance must be further studied at a chromosomal level to select the best targets for MAS. A comparison of the QTLs for yield performance and stability, together with QTLs coming out of this work, is in progress. This will improve our knowledge of the genetic control, biology and physiology of drought response and tolerance, and will allow us to identify the markers usable in a breeding program focused on an improvement of drought tolerance in the *Triticeae*.

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