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ORIGINAL PAPER

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Identification of drought-inducible genes and differentially expressed sequence tags in barley

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Abstract Drought limits cereal yields in several regions of the world and plant water status plays an important role in tolerance to drought. To investigate and understand the genetic and physiological basis of drought tolerance in barley, differentially expressed sequence tags (dESTs) and candidate genes for the drought response were mapped in a population of 167 F_8 recombinant inbred lines derived from a cross between "Tadmor" (drought tolerant) and "Er/Apm" (adapted only to specific dry environments). One hundred sequenced probes from two cDNA libraries previously constructed from drought-stressed barley (*Hordeum vulgare* L., var. Tokak) plants and 12 candidate genes were surveyed for polymorphism, and 33 loci were added to a previously published map. Composite interval mapping was used to identify quantitative trait loci (QTL) associated with drought tolerance including leaf relative water content, leaf osmotic potential, osmotic potential at full turgor, water-soluble carbohydrate concentration, osmotic adjustment, and carbon isotope discrimination. A total of 68 QTLs with a limit of detection score ≥2.5 were detected for the traits evaluated under two water treatments and the two traits calculated from both treatments. The number of QTLs identified for each trait varied from one to 12, indicating that the genome contains multiple genes affecting different traits. Two candidate genes and ten differentially expressed sequences were associated with QTLs for drought tolerance traits.

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Introduction

Barley (*Hordeum vulgare* L.) is a model species for genetic and physiological studies and shows a wide range of adaptations to various habitats. It is an annual, diploid self-pollinating species with a relatively short life cycle. Primitive landraces and the wild progenitor of barley (*H. spontaneum*) exhibit large variations in physiology, morphology and genetics, which might be used to improve cultivated barley (Nevo 1992; Forster et al. 2000).

Abiotic stresses including drought can significantly reduce crop yields and restrict the latitudes and soils on which commercially important species can be cultivated (Blum 1985). Identifying and understanding mechanisms of drought tolerance is crucial to the development of tolerant commercial cultivars. Thus, the responses of plants to various stresses have for decades been the focus of physiological studies (Levitt 1980) and of molecular genetics studies (Grover et al. 1999; Forster et al. 2000). A large and increasing number of genes, transcripts and proteins have been correlatively implicated in stress response pathways, while their precise functions in either tolerance or sensitivity remain unclear (Bray 1997). Extensive efforts have also been devoted to the characterization of genes induced or upregulated by drought (Close et al. 1989, 1993). Correlative evidence suggests a possible role for a number of these drought-induced genes in protecting cells from the harmful effects of dehydration (Bray 1997; Close 1997; Xu et al. 1996).

Drought stress tolerance is a quantitatively inherited trait, controlled by several genetic loci (QTL). Tolerance to drought involves a complex of mechanisms working in combination to avoid or tolerate water deficits. Water deficit occurs when water potentials in the rhizosphere are sufficiently negative to reduce water availability to

sub-optimal levels for plant growth and development. When plants are subjected to drought stress, a number of physiological responses have been observed (Ludlow and Muchow 1990; Fukai and Cooper 1995). In some cultivated cereals, osmotic adjustment (OA) has been found to be one of the physiological mechanisms associated with plant resistance to water deficit (Morgan 1984; Blum 1988; Zhu et al. 1997). Relative water content (RWC) is a measure of plant water status resulting from a cellular water deficit. While total water content is a useful indicator of water transport in the soil-plant-atmosphere continuum, it does not account for OA. Hence RWC is an appropriate estimate of plant water status as affected by leaf water potential and OA (Barrs and Weatherley 1962). Carbon isotope discrimination is another method used to screen for drought tolerance. The correlation between water use efficiency and carbon isotope discrimination has been extensively studied in several crops including wheat (Farquhar and Richards 1984; Condon et al. 1990) and barley (Acevedo 1993).

Physiological, morphological and developmental changes that confer drought tolerance in plants must have a molecular genetic basis. Identification and genetic mapping of QTL for specific drought tolerance traits combined with the mapping of candidate genes is a useful approach to dissecting the genetic basis of drought tolerance. Large expressed sequence tag (ESTs) databases have been obtained at different developmental stages from tissues and organs of plants exposed to a variety of environmental conditions. Mapping of differentially expressed sequences can be useful for the identification of candidate genes controlling important traits. If validated with accurate phenotyping and properly integrated in marker-assisted breeding programs, this approach can facilitate gene pyramiding and accelerate the development of drought tolerant genotypes.

Genetic linkage maps based on molecular markers have been developed for most crop species. These maps have been important in a number of applications in plant science including the location of genetic loci that control many important traits, map-based gene cloning, comparative mapping, and marker-assisted selection (MAS) in breeding. Perhaps the most profound impact of these approaches has been the study of quantitatively inherited traits. The concept of using genetic markers to identify specific regions of the genome has been well established using morphological characters, but developments in molecular biology have increased the repertoire of polymorphic assay procedures available. Linkage maps containing large numbers of RFLP, RAPD, AFLP and STS markers throughout the barley genome have been constructed by many workers (Graner et al. 1991; Heun et al. 1991; Hayes 1994). Molecular mapping of genes controlling plant resistance to drought stress has lagged behind that for disease resistance and morphological characters because of the complexity of drought tolerance and its association with many traits.

The objective of this study was to identify chromosomal locations and genetic contributions of drought-inducible genes and differentially expressed sequence tags (dESTs) related to drought tolerance in a barley population. In this context, an integrated genetic linkage map containing 166 loci was constructed and QTL affecting drought trait variation were identified. The results showed that drought-inducible genes and (dESTs) are associated with QTL that have an impact on drought tolerance in barley.

Materials and methods

Plant materials

The barley variety Tokak, known for its adaptation to the central region of Turkey, was used for cDNA library construction (Ozturk et al. 2002). For constructing a genetic linkage map and mapping QTL for drought traits, a segregating population of 167 barley recombinant inbred lines (RILs) derived from a cross between Tadmor (drought tolerant) and Er/Apm (adapted to specific environments) was used. Tadmor, a two-row variety selected from a Syrian landrace, is characterized by high yield stability (Grando 1989) and a high OA capacity (Teulat et al. 1997). Er/Apm, a two-row variety adapted to moderate rainfall conditions (Acevedo 1987), was developed by International Center for Agricultural Research in the Dry Areas (ICARDA, Syria). RILs were advanced by bulk until the F_4 generation at ICARDA and then by single-seed descent until the F_8 generation at CIMMYT (Mexico).

Probes and hybridization

Twelve candidate genes from different sources and 103 dESTs from barley (Ozturk et al. 2002) were used as probes to detect polymorphism between the two parents. The 50 probes detecting one or more polymorphisms were subsequently analyzed for segregation among the RIL population (Table 1). Survey membranes of parental DNA were prepared by digesting 20 μg aliquots of DNA with 20 U of each of the following restriction endonucleases: *Eco*R1, *Eco*RV, *Dra*I, *Xba*I, *Hin*dIII, *Bam*HI, *Pst*I, *Xho*I, *Hae*III, *Kpn*I and *Sca*I for approximately 16 h. The digested DNA was then separated on 0.9% (w/v) agarose gels with 0.5× TEA electrophoresis buffer, and was then transferred to Hybond N+ membrane (Amersham, Arlington Heights, Ill., USA) following the manufacturer's recommendations. Parental survey filters were hybridized with 12 candidate genes from different sources and 100 ESTs (constructed from the *H. vulgare* subspecies Tokak). The filters were then placed against X-ray film to obtain autoradiographs. Based on the results of the parental surveys, the appropriate DNA filters for the mapping population

Table 1 New genes mapped by Southern analysis in the barley (Tadmor/Er/Apm) RIL population

Probes (GenBank number)	Gene product	Restriction enzyme	Species
AF519805	(TapK3) Serine-threonine protein kinases	*Dra*I	Wheat
BM816600	Light inducible protein ATLS1	*Dra*I	Barley
BM816570	Leucyl aminopeptidase	*Eco*RI	Barley
BM816268	Ubuquitin-protein ligase UBC9	*Dra*I	Barley
BM816351	Sterol methyl oxidase	*Eco*RI	Barley
BM816947	EIN2 protein	*Dra*I	Barley
BM816370	Alcohol dehydrogenase	*Hin*dIII	Barley
BM817329	Hexaprenyldihydroxybenzoate methyltransferase	*Hin*dIII	Barley
BM815937	Blue copper-binding protein	*Xba*I	Barley
BM815931	Hypothetical protein K3 M16_30 (Arabidopsis)	*Xba*I	Barley
BM816242	Glutathione S-transferase 1	*Hin*dIII	Barley
BM816463	Blue copper-binding protein	*Dra*I	Barley
BM816306	Oxalate oxidase	*Dra*I	Barley
BM816474	Cathepsin B	*Eco*RI	Barley
BQ740141	Putative kinetochore protein	*Bam*HI	Barley
BQ740142	Vacuolar H + -ATPase proteolipid 16 KD	*Dra*I	Barley
BM816122	*C. elegans* predicted protein	*Eco*RI	Barley
BM817178	Isocitrate dehydrogenase	*Dra*I	Barley
BM816381	Endopeptidase Clp	*Dra*I	Barley
BQ740145	S-adenosyl-L-homocysteine hydrolase	*Xba*I	Barley
BM816303	Aminomethyltransferase	*Dra*I	Barley
BM816398	Late-embryogenesis abundant protein	*Dra*I	Barley
BM816122	Hypothetical protein C18B2.4	*Dra*I	Barley
BM816685	FPF1 protein	*Dra*I	Barley

were generated and used to obtain segregation data for the RFLP probes that detected parental polymorphisms (Table 1). The probes were denatured for 10 min and radioactively labeled by random priming using [^{32}P]-dCTP. A volume of 11 µl oligonucleotide mixture, 2 µl Klenow, and 3 µl [^{32}P] were added to 100 ng of DNA probe. The mixture was incubated at 37°C for 2 h and denatured for 10 min by adding 25 µl 0.4 N NaOH. Lambda *Hin*dIII DNA marker was added to the probes before hybridization. Prehybridization and hybridization processes were performed at 65°C using hybridization buffer containing 1 M Na_2PO_4, 20% SDS, 66.6 g/l bovine serum albumin and 10 mg/ml denatured salmon-sperm DNA. Three washing steps were conducted at 65°C using 2×, 1× and 0.5× SSC buffer for washing steps 1, 2 and 3, respectively. A volume of 10 g/l SDS was added to each of the washing buffers.

Measurement of traits

The details of the experimental design and conditions were described in Teulat et al. (2001) and the analyses in the present study utilized those data as well as those from the field study by Teulat et al. (2002). Briefly, the experiments covered nine blocks in a random incomplete block design. Two sets of means were considered: line means for the total experiment and line means for the first four blocks. The adjusted means generated by fixing the block were used in this study. Two sets of plants were grown under two water regimes; one set under water deficit (s) where plants were at 14% of field capacity and the other set with an irrigated supply (I) at 100% field capacity. Water stress was imposed at the four-leaf stage by stopping the irrigation for the first set of the plants. Leaf RWC was measured as described in Barrs and Weatherley (1962). In order to avoid interaction between OA and growth, RWC was measured on the last fully expanded leaf (Munns 1988; Li et al. 1993). The leaf osmotic potential (OP) was measured using a freezing-point micro-osmometer (Roebling 13 GS/IS). Osmotic potential at full turgor (OP_{100}) was calculated according to Wilson et al. (1979). The water-soluble carbohydrate concentration (WSC) and the WSC at full turgor (WSC_{100}) were determined according to Dubios et al. (1956) from 100 mg of fresh leaf. OA was then calculated according to Ludlow et al. (1983). The accumulation of WSC at 100% RWC (full turgor) was then calculated (Teulat et al. 2001).

In addition, carbon isotope discrimination (Δ) was measured by isotope mass spectrometry on mature grains from field-grown plants harvested in 1999 (field experiments conducted in Montpellier, France, under rain-fed conditions and with irrigation supply). Details of the experimental design and climatic conditions were described in Teulat et al. (2002).

Genetic map construction and QTL detection

The 50 clones that showed polymorphism between the parental lines were used to probe the DNA blots from the 167 RILs. For each marker the RILs were scored as '3' for presence of the parental band of the female parent (Tadmor), '1' for the male parent (Er/Apm) or '0' for missing data. Linkage analysis and map construction were performed by using Map Manager QTX14 (Manly and Cudmore 1997) using the Kosambi function to

convert the recombination frequencies to centiMorgans (cM). The linkage groups were constructed using the "make linkage group" command with a minimum limit of detection (LOD) score of 2.0 followed by the ripple command for each linkage group to check the final order of markers. Order information from the previously published map (Teulat et al. 2002) was also considered in some regions. The phenotypic data for the eight traits were analyzed by composite interval mapping (CIM) analysis (Zeng 1994). The QTL Cartographer program (Basten et al. 2000) was used to analyze the data with 20 markers as a background control and a window size of 2 cM. This method uses a multiple regression procedure adjusting for background effects of markers (co-factors) other than those in the interval being tested. A 1,000 permutation test indicated that a LOD threshold level of 2.5 was appropriate for significant LOD scores for CIM.

Results and discussion

Parental polymorphism and genetic linkage map

Of the 112 probes used, 50 (45%) detected polymorphisms between the parental lines for at least one of the 11 restriction enzymes used. The autoradiographs of 12 probes could not be scored, 14 probes identified loci that were unlinked and 24 probes identified 33 loci that were merged with 133 previously mapped markers to produce a map with 166 loci in seven linkage groups (Fig. 1). The linkage groups ranged from 216 to 407 cM with an average distance of 12.2 cM between loci. The map orientation was based on the previous linkage map developed by Teulat et al. (2002). In general, there was good agreement for the marker order between this map and previous published maps (Teulat et al. 1998, 2001, 2002; Becker and Heun 1995); however, the new markers expanded the genome coverage substantially. The chromosomes with the most markers were chromosomes 2H and 5H with 28 markers each, followed by 7H (25 markers), 3H (24 markers) 1H (23 markers), and 6H with 20 markers.

QTL analysis

The population used in this study was useful for identifying loci controlling quantitative traits under drought stress because it was derived from a cross between genetically diverse parents exhibiting contrasting traits for drought tolerance. Significant associations between molecular markers and putative QTL that were analyzed for the 167 barley RILs were detected for all traits that were measured by Teulat et al. (2001, 2002) on all chromosomes (Fig. 1). A total of 68 QTLs with LOD scores ≥2.5 were identified. Multiple QTLs were found for almost all traits, many of which were overlapping (Fig. 1). One to 12 QTLs were identified for each trait, indicating that the genome contains multiple genes affecting these traits. The highest LOD peak (7.9) of the study was obtained for osmotic potential under irrigation (OP_i) on chromosome 2H and for WSC_{100} under drought stress (WSC_{100s}) on chromosome 4H (Table 2).

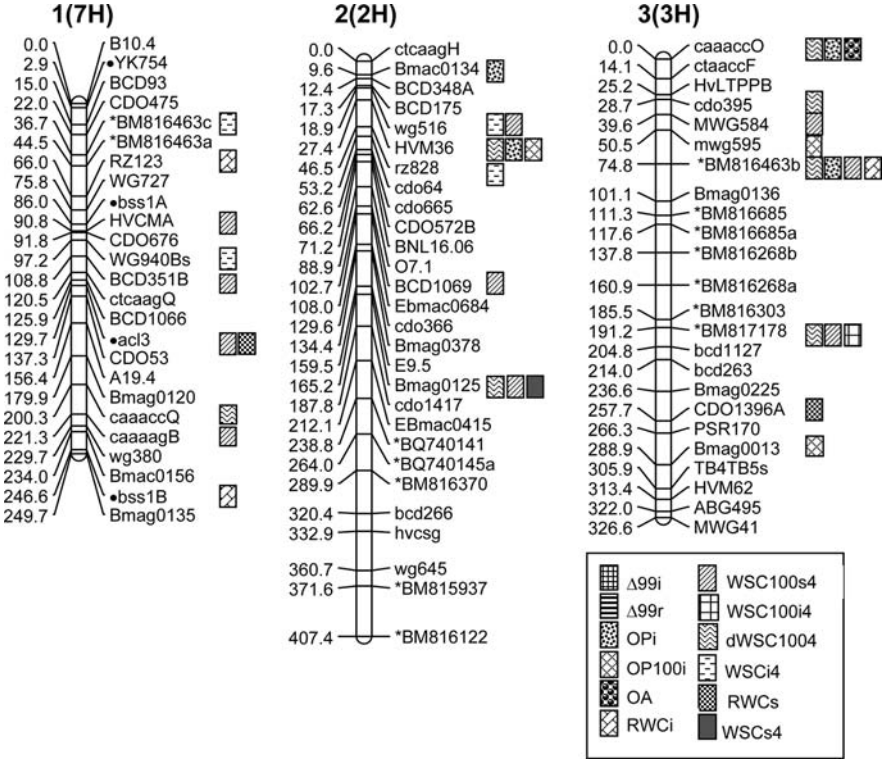

Fig. 1 Genetic linkage map of the Tadmor × Er/Apm barley RIL population showing QTL positions for OP, OP_{100}, WSC, WSC_{100}, accumulation of WSC at 100%RWC ($dWSC_{100}$), OA, RWC and carbon isotope discrimination (Δ). *s* Water stress treatment, i.e. the plants were at 14% of field capacity, *i* irrigated plants (100% of field capacity), *4* trait was measured for the four first blocks only (85 RILs), *r* trait was measured under rainfed conditions. Markers preceded by an *asterisk* are ESTs and genes are represented by *filled circles*

QTL for osmotic adjustment, osmotic potential and relative water content

Osmotic potential, OP_{100} and RWC are components of OA and are highly correlated (Teulat et al. 1997) because OA is calculated using OP_{100} for the two water treatments (OP_{100i}, OP_{100s}). For that reason, OA is considered to be a drought-related trait (Teulat et al. 2001). Several chromosomal regions related to variation in OA and water status were detected. Two QTL were identified for OA, one on chromosome 3H and one on 5H. For RWC, six QTL were detected under irrigation treatment and three were detected under conditions of water stress. Seven QTL were identified for OP in the irrigated group and three were detected under conditions of water stress. QTLs for OP_{100} were also detected for both treatments. A total of seven QTL were detected on all chromosomes except 7H. One of them was identified under drought stress and six under irrigated conditions. OA has been mapped in rice (Zhang et al. 1999; Lilley et al. 1996), wheat (Morgan and Tan 1996) and barley (Teulat et al. 1998, 2001; Qingyang et al. 2001), and the comparative mapping of such QTLs may provide new information on shared genetic variation for that trait among cereals.

QTL for water-soluble carbohydrate and related traits

Thirteen QTLs for WSC were detected. Nine were detected under irrigation, two each on chromosomes 2H, 5H, and on 7H and three on 4H. Four QTLs were identified under stress conditions (one on 2H and 1H and two on 5H). In addition, 13 QTLs were detected for (WSC_{100}), two under irrigated conditions and 12 under water stress conditions, one QTL on chromosome 3H being detected for both treatments. Nine QTLs for accumulation of WSC at 100%RWC ($DWSC_{100}$) were also identified. One QTL was found on chromosomes 7H, 4H and 5H, two on 2H and four on 3H. In Teulat et al. (2001), only one QTL was identified for WSC under water stress on chromosome 2H. They reported no significant QTL for WSC under irrigated conditions but a QTL was detected for WSC_{100} on chromosome 4H.

QTL for carbon isotope discrimination

Two QTL for carbon isotope discrimination under irrigated field conditions were detected in addition to two QTL under rainfed conditions. In Teulat et al. (2002) only one QTL was detected in the irrigated treatment group but four QTL were found when the analyses were performed across the two treatment groups. Among them, the two QTL identified under rainfed conditions were on chromosomes 1H and 5H (Table 2).

The larger number of QTL identified in this work was mainly due to the larger number of mapped markers, which enhanced the map, and to the use of CIM. CIM is a combination of simple interval mapping and multiple linear regression that uses other markers to control for genetic background. However, in this study the percentage of variation explained by each individual QTL is quite low, ranging from 4% up to 17%.

Correlation between traits

Genomic regions were found where QTL for different traits overlapped. The genomic regions with the most overlapping traits were caaaccO and BM816463b, on chromosome 3H and HVM36 on chromosome 2H. For example, QTL for OA, OP and $DWSC_{100}$ were all mapped to approximately the same chromosomal location around caaaccO. Similarly, QTL for $DWSC_{100}$, RWC, OP and WSC_{100} overlapped in the same genomic region (BM816463b). The co-location of the $DWSC_{100}$ and RWC QTL in more than one genomic region, HVM36 on chromosome 2H and BM816463b on 3H, under water stress suggests that the accumulation of WSC may be important for plants to maintain their RWC. Considering confidence intervals around QTL position estimation, these chromosomal regions could be considered to be similar. Overlapping QTL were also found on the short arm of chromosome 4H (OP_{100}, WSC and carbon isotope discrimination) and the long arm of 1H (OP, WSC and carbon isotope discrimination).

The distinction between linkage and pleiotropy is important for breeding purposes, especially if both desirable and undesirable traits are associated with the same locus or QTL region. In the present study, QTL for OA and $DWSC_{100}$ were positively associated and mapped to the same region (caaaccO) on chromosome 3H. Physiologically, these traits are components of drought tolerance, therefore, the co-localization of these QTL is most likely due to pleiotropic effects of the same gene(s).

Differently expressed genes co-segregating with drought related traits

The co-location of dESTs and candidate genes under drought stress with QTLs suggests that they may be involved in a drought tolerance mechanism, however, further research is needed for verification. In this study, among the 68 QTLs identified, 19 were co-located with ESTs or genes at different 12 loci. Ten dESTs and two candidate genes were found to be associated with different QTLs for drought-related traits (Fig. 1 and Table 2).

The locus BM816463b on chromosome 3H coding for blue copper-binding protein co-segregated with QTLs for RWC, WSC_{100}, OP and $DWSC_{100}$. A gene coding for the same protein has been isolated from *Arabidopsis*, which suppressed aluminum absorption in roots (Ezaki et al. 2001). Aluminum toxicity causes cell damage similar to that induced by drought stress (Yang et al. 2002). It has been reported that exposure of plants to various environmental perturbations, including intense

Table 2 Significant QTLs for drought-related traits detected by CIM using Tadmor × Er/Apm barley RIL population. *s* Water stress treatment, i.e. plants were at 14% of field capacity, *i* irrigated plants (100% of field capacity), *4* trait was measured for the four first blocks only (85 RILs), *r* trait was measured under rainfed conditions. For Δ, r refers to 390 mm of rainfall whereas i refers to 98 mm additional water supply

Trait	Chromosome	Nearest marker	Position (cM)	LOD score	Additive effect	R^2
RWC_s	1(7H)	Acl3	129.7	3.1	0.870	0.05
RWC_s	3(3H)	CDO1396A	257.7	3.0	−0.894	0.05
RWC_s	6(6H)	BCD1	202.5	3.3	1.073	0.08
OP_s	4(4H)	CtaaccD	126.8	2.8	−0.033	0.05
OP_s	4(4H)	BM816306a	220.2	3.3	0.041	0.08
OP_s	7(5H)	MWG502	0.0	3.1	0.034	0.06
OP_{100s}	4(4H)	CDO795	42.2	2.7	0.018	0.09
RWC_i	1(7H)	RZ123	68.0	2.9	0.260	0.07
RWC_i	1(7H)	Bss1B	246.6	3.5	−0.254	0.07
RWC_i	3(3H)	BM816463b	80.8	2.6	0.303	0.09
RWC_i	7(5H)	E9.1	109.2	4.3	−0.393	0.15
RWC_i	7(5H)	WG889B	135.7	2.7	0.243	0.05
RWC_i	7(5H)	CDO484	356.5	3.0	0.267	0.08
OP_i	2(2H)	Bmac0134	9.7	3.2	0.017	0.04
OP_i	2(2H)	HVM36	31.5	7.9	−0.030	0.16
OP_i	3(3H)	CaaaccO	0.0	2.8	0.014	0.04
OP_i	3(3H)	BM816463b	74.8	3.8	−0.020	0.08
OP_i	5(1H)	CDO202	246.1	2.9	0.015	0.05
OP_i	7(5H)	CaaaccS	17.3	2.8	0.021	0.08
OP_i	7(5H)	BM816381b	42.6	3.3	−0.018	0.06
OP_{100i}	2(2H)	HVM36	37.5	3.6	−0.013	0.08
OP_{100i}	3(3H)	MWG595	70.5	6.8	−0.017	0.15
OP_{100i}	3(3H)	Bmag0013	302.9	4.3	−0.011	0.07
OP_{100i}	5(1H)	CDO202	246.1	4.6	0.011	0.06
OP_{100i}	6(6H)	EBmac0806	146.0	3.0	0.008	0.04
OP_{100i}	7(5H)	BM816381a	42.0	3.4	−0.011	0.06
OA	3(3H)	CaaaccO	0.0	3.7	0.034	0.07
OA	7(5H)	CaaaccS	9.3	3.9	0.037	0.08
WSC_{s4}	2(2H)	Bmag0125	175.2	4.8	−51.885	0.15
WSC_{s4}	5(1H)	Bpc	242.8	4.3	41.232	0.09
WSC_{s4}	7(5H)	BM816242	67.5	2.9	40.662	0.10
WSC_{s4}	7(5H)	BM816122a	226.2	2.8	48.022	0.13
WSC_{i4}	1(7H)	BM816463c	36.7	2.8	9.742	0.06
WSC_{i4}	1(7H)	WG940Bs	97.2	2.7	−9.091	0.05
WSC_{i4}	2(2H)	WG516	20.9	3.7	11.155	0.07
WSC_{i4}	2(2H)	RZ828	46.5	4.2	−11.548	0.08
WSC_{i4}	4(4H)	CDO669	2.5	3.3	9.425	0.06
WSC_{i4}	4(4H)	BM816474b	159.5	4.2	12.310	0.09
WSC_{i4}	4(4H)	BM816306a	218.2	2.9	−9.895	0.05
WSC_{i4}	7(5H)	CDO400	266.7	3.4	10.042	0.06
WSC_{i4}	7(5H)	CDO484	358.5	3.6	−11.108	0.08
WSC_{100s4}	1(7H)	HVCMA	90.8	3.6	−30.836	0.05
WSC_{100s4}	1(7H)	BCD351B	114.8	2.7	28.935	0.06
WSC_{100s4}	1(7H)	Acl3	129.7	3.8	28.122	0.06
WSC_{100s4}	1(7H)	CaaaagB	227.3	2.9	22.172	0.04
WSC_{100s4}	2(2H)	WG516	24.9	2.9	−24.187	0.05
WSC_{100s4}	2(2H)	BCD1069	102.7	3.5	−34.292	0.05
WSC_{100s4}	2(2H)	Bmag0125	175.2	4.8	−35.383	0.11
WSC_{100s4}	3(3H)	MWG584	43.6	3.9	−28.941	0.07
WSC_{100s4}	3(3H)	BM816463b	84.8	5.9	60.598	0.26
WSC_{100s4}	3(3H)	BM817178	191.2	5.7	−35.935	0.09
WSC_{100s4}	4(4H)	Bmag0353	86.9	7.9	41.720	0.12
WSC_{100s4}	7(5H)	CDO400	266.7	4.2	27.687	0.07
WSC_{100i4}	3(3H)	BM817178	203.2	3.5	10.564	0.11
WSC_{100i4}	4(4H)	CDO669	4.5	4.8	13.249	0.17
$DWSC_{100.4}$	1(7H)	CaaaccQ	206.3	3.2	33.711	0.08
$DWSC_{100.4}$	2(2H)	HVM36	27.5	2.8	−26.608	0.05
$DWSC_{100.4}$	2(2H)	Bmag0125	181.2	2.6	−32.759	0.08
$DWSC_{100.4}$	3(3H)	CaaaccO	4.0	4.5	37.343	0.10
$DWSC_{100.4}$	3(3H)	CDO395	38.7	3.6	−32.760	0.08
$DWSC_{100.4}$	3(3H)	BM816463b	76.8	3.2	41.765	0.11
$DWSC_{100.4}$	3(3H)	BM817178	191.2	3.7	−35.232	0.08
$DWSC_{100.4}$	4(4H)	Bmag0353	86.9	6.5	42.179	0.13
$DWSC_{100.4}$	7(5H)	BM816474a	262.0	4.2	40.155	0.12

Table 2 (Contd.)

Trait	Chromosome	Nearest marker	Position (cM)	LOD score	Additive effect	R^2
$\Delta 99_r$	5(1H)	ABC155	248.5	4.0	−0.153	0.09
$\Delta 99_r$	7(5H)	Bmag0223	197.6	2.8	0.172	0.12
$\Delta 99_i$	4(4H)	WG876	22.1	3.4	0.121	0.06
$\Delta 99_i$	7(5H)	Bmac0113	116.1	2.6	0.106	0.05

light, drought, temperature stress, the presence of metal toxicity (e.g., aluminum), can lead to the generation of activated oxygen species (AOS). These AOS in turn generate hydroxyl radicals, which can affect a variety of biological macromolecules (Halliwell and Gutteridge 1984; Kampfenekel et al. 1995). For example, AOS can react with unsaturated fatty acids in the plasma membrane to break down essential membrane lipids, which may lead to severe cellular damage.

The loci BM816306a, BM816242, BM816474b, BM817178, BM816381(a, b) and BM816122a coding respectively for oxalate oxidase, glutathione S-transferase 1, cathepsin B, isocitrate dehydrogenase, endopeptidase Clp, and hypothetical protein C18B2.4 (Ozturk et al. 2002) co-segregated with several QTL for $DWSC_{100}$, OP, RWC, WSC and WSC_{100}. The role of most of these genes in drought tolerance is not yet known. However, some studies report a role for a few of these genes in biotic and abiotic stresses. For example, Plaisance and Gronwald (1999) reported that glutathione S-transferases (plant cell proteins believed to play an important role in protecting plants from various environmental stresses such as plant diseases and pesticides) attach lipid hydroperoxides, which are harmful compounds that are generated in plants exposed to various stresses such as drought and pathogen invasion. Two glutathione S-transferase proteins have been purified from sorghum that have the ability to detoxify lipid hydroperoxides (Plaisance and Gronwald 1999). These proteins might play an important role in protecting plants from toxic lipid hydroperoxides that are formed under drought stress conditions.

Genes co-segregating with drought traits

Two genes co-segregated with QTL for RWC and WSC_{100}. The *Acl3* locus on chromosome 7H, which is associated with QTL for RWC and WSC_{100} codes 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 fatty acid synthetase involved in the de novo synthesis of the fatty-acyl chain, especially in chloroplasts. This gene could have a role in the protection of membranes or in membrane fluidity during stress.

The gene *bSS1B*, coding for sucrose synthase, (Sanchez de la Hoz et al. 1992), co-segregated with a QTL for RWC. The enzyme sucrose synthase is a key enzyme in carbohydrate metabolism, catalyzing the reversible conversion of sucrose uridine-diphosphate into fructose and UDP-glucose (Kleines et al. 1999). Synthesis of sugars or compatible solutes has widely been observed as a mechanism that may help plants cope with water deficit (Whittaker et al. 2001). QTLs for RWC were previously identified in chromosomal regions containing mapped genes (Teulat et al. 1998, 2001). Among the results, co-segregation of RWC QTLs under water stress treatment was observed with the dehydrin gene, *dhn*4, on chromosome 6H. Dehydrins are one of the families of proteins that are synthesized in plants in response to dehydration, low temperature, osmotic stress, seed drying, and exposure to abscisic acid. Eleven barley *Dhn* genes have been identified and sequenced (Close et al. 1989). Inheritance studies, including QTL analysis in several crop plants have reported co-segregation of *Dhn* genes with phenotypes associated with drought and freezing stresses. The first such observation was in barley, where a cluster of *Dhn* loci (*Dhn*1, *Dhn*2 and *Dhn*4a) overlapped a major QTL for winter hardiness in a winter (Dicktoo) by spring (Morex) dihaploid mapping population on chromosome 5H (Pan et al. 1994).

Numerous QTL mapping studies examining drought tolerance and related traits in maize, rice, barley and wheat have demonstrated that drought tolerance is affected by several loci, each of which have relatively small effects (McCouch and Doerge 1995; Quarrie 1996). Many genes may be involved in drought tolerance mechanisms and the identification of several QTL involved in drought or dehydration tolerance in rice (Lilley et al. 1996), maize (Lebreton et al. 1995) or in sorghum (Borrell et al. 1999) 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 the tolerance mechanisms, but for many of these genes no direct role in tolerance has been clearly demonstrated. Some of the QTL 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 QTL involved in adaptation could give some information on their role as well as direction for further research.

Additional QTL studies are useful for identifying the chromosomal regions that are more consistently associated with drought tolerance. The practical application of the identified QTL is for marker-assisted selection aimed at improving drought resistance in barley. Also, research results from barley can be used to explore

particular drought resistance mechanisms in other cereals. Further studies involving fine mapping of genomic regions associated with drought resistance across genetic backgrounds or species will be required for cloning of genes controlling resistance to drought in plants.

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