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Full Length Research Paper

Drought - inducible genes and differentially expressed sequence tags associated with components of drought tolerance in durum wheat

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Drought stress is one of the most important environmental factors reducing cereal yields. The genetic and physiological bases of drought tolerance in durum wheat was investigated by QTL mapping and by mapping candidate genes derived from differentially expressed genes and previous investigations. A recombinant inbred population derived from a cross between two durum (*Triticum turgidum* L. var durum) parents Jennah Khetifa and Cham1 that exhibit contrasting traits for drought tolerance was employed. Twelve known genes and 103 differentially expressed sequence tags (dESTs) were surveyed and 55 detected polymorphism between the two parental lines. In total, 162 loci including 6 known genes, 37 dESTs, and an additional 119 markers that were unlinked in the previous map have been merged with 306 previously mapped markers to produce a new map with 468 loci. Single point analysis and composite interval mapping were used to identify the genomic regions controlling traits related to drought stress. Significant QTL were identified for canopy temperature, photosynthesis-related parameters and water status index. One hundred and seventy eight markers, including 6 candidate genes and 19 differentially expressed sequences were associated with QTL for drought tolerance traits. The results indicate that there is considerable potential for improving drought tolerance of durum wheat by using marker-assisted selection.

Key words: Durum wheat, drought tolerance, QTL analysis, genetic linkage mapping, ESTs, RFLP, marker-assisted selection.

INTRODUCTION

Drought is one of the most common environmental stresses worldwide that affects growth and development of plants through alternations in metabolism and gene expression (Leopold, 1990). Despite many decades of research, drought continues to be a challenge to agricultural scientists in general and to plant breeders in particular. This is due to the unpredictability of its occurrence, severity, timing and duration; and to the interaction of drought with other abiotic stresses (Ceccarelli and Grando, 1996). An understanding of the genetic and physiological basis of drought tolerance would facilitate the development of improved crop management and breeding techniques and lead to improved yield in unfav-orable environments. Durum wheat (*Triticum turgidum* L. var durum) is an allotetraploid (genome AABB, 2n = 4X = 28) with seven homoeologous groups.

Traits related to water use efficiency and photosynthesis related parameters have been found to play a major role in drought tolerance in durum wheat (Nachit et al., 2000; Araus et al., 1997; Rekika et al., 1998). There is no direct and simple strategy for characterizing QTL affecting drought tolerance traits. Map-based cloning can more readily be applied to small genome species, such as *Arabidopsis,* rice or tomato. The mapping and analysis of candidate genes can be a useful approach to identifying genes affecting drought tolerance. As discussed by De Vienne et al. (1999), the selection and validation of

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candidate genes may rely on two non-exclusive approaches. The first one, the "functional" candidate gene approach, is based on the *a priori* choice of gene(s) that may be functionally related to the trait. A correlation between the trait under study and allelic polymorphism of the candidate, regardless of the genetic background, is evidence in support of the candidate gene. The second approach, the "positional" candidate gene approach, relies on QTL mapping and on examination of known-function genes or sequences that map in the same region, the effect of which may be related to the trait. Sequenced cDNAs from a tissue-specific library (that is, tissues that express the desired trait) can be identified and mapped, and potential candidate genes can then be identified in conjunction with QTL mapping.

The objective of this study was to identify chromosomal locations and genetic contributions of genes controlling traits related to drought tolerance in durum wheat. In this context, positional and functional candidate gene approaches were combined to detect and to begin the validation of candidate drought genes in durum wheat. Candidate drought genes and differentially expressed barley sequences were used to produce an integrated genetic linkage map containing 468 markers. The results indicated that drought inducible genes and differentially expressed sequence tags were associated with QTLs controlling traits that are related to drought tolerance in durum wheat. Locating parts of the genome that contribute to drought tolerance in durum by the use of molecular mapping and candidate gene approaches promises to increase our understanding of drought resistance and generate markers for strategic improvement of durum wheat by marker-assisted breeding.

MATERIALS AND METHODS

Plant materials

A durum population of 110 F_9 recombinant inbred lines (RILs) derived by single-seed descent from the cross ICD-MN91-0012 between Tamgurt (Jennah Khetifa) and Cham 1 was employed. The CIMMYT/ICARADA durum-breeding program for Mediterranean dryland developed the population at the Tel Hadya research station (Aleppo province, Syria). Jennah khetifa (JK), a landrace variety, is characterized by moderate resistance to drought and shows specific adaptation to North African continental dryland. Cham 1 is grown for commercial production in several countries of the Mediterranean basin.

Growing conditions and treatments

The RILs trials were grown in 1996/97 at the Tel Hadya research station (TH) 35 Km south west of Aleppo city/Syria, at 284 m above sea level. To create different environmental conditions, a stress-screening methodology that simulates different environments at one site (Nachit, 1983; Nachit and Ketata, 1986) was used in this study. The first environment was Tel Hadya rainfed (RF), characterized by moderate drought stress. The date of sowing was November 15th and the date of harvest was June 15th. The second environment was Tel Hadya late planting (LP), characterized by severe drought stress. The sowing date was April 1st and the harvest date was June 15th. Fertilizers applied at sowing date were 60 Kg/ha nitrogen unit (NH_4NO_3) and 40 kg/ha of P_2O_5 for both RF an LP. In contrast to LP and RF environments, the third environment was Tel Hadya irrigated (IR) and an additional 30 kg/ha of N fertilization and supplementary irrigation of 50 mm were applied.

Experimental design

The 110 RILs were divided over 6 blocks where 19 RILs were included in each incomplete block, with five durum genotypes as checks (*Omrabi5, Haurani, Korifla, Cham1,* and *Gidara2*). The field design used was the augmented design (Federer, 1956; Peterson, 1985). The total number of entries of the whole trial was 152 (including 110 RILs, the 2 parents and the 5 checks repeated in each block).

Measurements of traits

The parents and RILs were evaluated for twelve traits related to drought tolerance as follows: Leaf relative water content (RWC) was measured as described in Barrs and Weatherley (1968) using the equation RWC (%) = [(W-DW) / (TW-DW)] x 100. Where, W: sample fresh weight, TW: sample turgid weight, DW: sample dry weight. To evaluate leaf osmotic potential (OP) values, the leaf tissue was cut, wrapped in aluminum foil, frozen in liquid nitrogen and stored at –20^{o}C until measurement. Osmotic potential was measured using a freezing-point micro-osmometer (Roebling 13 GS/IS, Germany). Relative water content and osmotic potential were then used to calculate the leaf osmotic potential at full turgor (OP100) as described by Wilson et al. (1997) using the equation OP100 = OP X (RWC –B)/(100-B) where B is the apoplastic water (Tetlow and Farrar, 1993). Osmotic adjustment (OA), which is a mechanism of conserving cellular hydration under drought stress, was calculated according to Ludlow et al. (1983). Water index (WI) was calculated as described by Araus et al. (2000) as the ratio between the reflectance at 970 nm (sensitive to plant water content) and the reflectance at 900 nm (a reference wavelength in which the absorption by water is null). Canopy temperature depression (CTD) was measured as described by (Nachit and Ketata, 1991) as the difference between air temperature and the average temperature of the leaf canopy. Carbon isotope discrimination, the ratio of stable carbon isotopes ($^{13}C/^{12}C$) in the plant dry matter compared to the value of the same ratio in the atmosphere, was measured by isotope mass spectrometry from mature grains (CID-G) and from leaf (CID-L) according to Araus et al. (1997). Fluorescence index (Fluo) was measured according to (Havaux et al., 1988; Ernez and Lannoye, 1991). Photosynthetically Active Radiation (PAR), the number of moles of photons in the radiant energy between 400 nm and 700 nm, was measured as described by Araus et al. (2000). It is a parameter relating the available visible solar radiation to the absorption by chlorophyll. The quantum yield (Q), which is a measure of photosynthetic efficiency expressed in moles of photons absorbed per mole of CO_2, and chlorophyll content (CHL), using a remote sensing technique was measured as described by Araus et al. (2000). Transpiration efficiency (TRS), the rate of photosynthetic assimilation per unit of transpired water was also measured according to Araus et al. (2000) and Royo et al. (2000).

Plant DNA

Fresh tissue from each of the parental lines and the 110 F9 RILs was collected and ground in liquid nitrogen. Extraction buffer (pH 7.8 - 8.0) containing 5 M NaCl, 1M Tris-HCL, 0.25 M EDTA, 20% SDS and 3.8 g/l sodium bisulfite was added to the tissue and incu-

Table 1. Probes mapped in durum (JK/Cham1) RIL population.

Probes GenBank #	Gene product or annotation	Restriction enzyme	Species	Reference
AF210723	Fructan 6-fructosyltransferase	XbaI	Barley	Sprenger et al., 1995
BM816620	Glycine dehydrogenase	DraI	Barley	Ozturk et al., 2002
BM816848	Unknown	DraI	Barley	Ozturk et al., 2002
BM816286	Unknown	XbaI	Barley	Ozturk et al., 2002
BM816268	Ubiquitin-conjugating enzyme E2	HindIII	Barley	Ozturk et al 2002
BM816248	Glutathione transferase	DraI	Barley	Ozturk et al., 2002
BM816648	Arginine decarboxylase	DraI	Barley	Ozturk et al., 2002
BM816904	Unknown	EcoRI	Barley	Ozturk et al., 2002
BM816571	Metallothioneine	BamHI	Barley	Ozturk et al., 2002
BM815987	Jasmonate induced protein	DraI	Barley	Ozturk et al., 2002
BM816940	Acyl carrier protein III precursor	BamHI	Barley	Ozturk et al., 2002
BM816953	Nitrilase-like protein	DraI	Barley	Ozturk et al., 2002
BM816618	Glycine dehydrogenase	XbaI	Barley	Ozturk et al., 2002
BM816624	Arginine 2-monooxygenase	DraI	Barley	Ozturk et al., 2002
BM815936	ORF107a, Arabidopsis	DraI	Barley	Ozturk et al., 2002
HC105A03	No annotation	HindIII	Barley	Ozturk et al., 2002
BM816579	Leucyl aminopeptidase	DraI	Barley	Ozturk et al., 2002
BM816370	Alcohol dehydrogenase	DraI	Barley	Ozturk et al., 2002
BM815946	Unknown (hypothetical protein, Arabidopsis)	DraI	Barley	Ozturk et al., 2002
BM816640	Unknown	XbaI	Barley	Ozturk et al., 2002
BM817327	4-alpha-glucanotransferase	DraI	Barley	Ozturk et al., 2002
BM815937	Blue copper-binding protein	DraI	Barley	Ozturk et al., 2002
BM815931	hypothetical protein K3M16_30	DraI	Barley	Ozturk et al., 2002
Probes GenBank #	Gene product	Restriction enzyme	Species	Reference
BM816242	Glutathione S-transferase 1	DraI	Barley	Ozturk et al., 2002
BM816257	actin depolymerizing factor 4	HindIII	Barley	Ozturk et al., 2002
BM817360	Sugar transporter	BamHI	Barley	Ozturk et al., 2002
BM816287	putative protein, Arabidopsis	DraI	Barley	Ozturk et al., 2002
BM816608	Glutathione oxidase	DraI	Barley	Ozturk et al., 2002
BM816153	Stearoyl-CoA desaturase	DraI	Barley	Ozturk et al., 2002
BM816306	Oxalate oxidase	DraI	Barley	Ozturk et al., 2002
BM816474	Cysteine protease	DraI	Barley	Ozturk et al., 2002
BM816609	Aluminum induced protein wali 5	DraI	Barley	Ozturk et al., 2002
BQ740214	No annotation	DraI	Barley	Ozturk et al., 2002
BM817222	G protein-coupled receptor	HindIII	Barley	Ozturk et al., 2002
BM816414	Early flowering protein 1	EcoRI	Barley	Ozturk et al., 2002
BM816121	Stearoyl-CoA desaturase	DraI	Barley	Ozturk et al., 2002
M96856	Protein associated with G-Box binding complex	DraI	Maize	de Vetten et al., 1992
D13042	Protein kinase	DraI	Arabidopsis	Koizumi et al., 1993
M94726	protein kinase	Dra1	Wheat	Anderberg and Walker-Simmons 1992
D13043	Cysteine proteinase	DraI	Arabidopsis	Koizumi et al., 1993
AF519805	Serine-threonine protein kinases	DraI	Wheat	Walker-Simmons 1997

Table 2. Distribution of molecular markers, assignment and centiMorgan (cM) coverage across the 14 durum A and B genome.

Chromosome	RFLPs	SSRs	AFLPs	SSPs	Genes	ESTs	Markers		cM	cM/Marker
							#	%		
1A	14	2	15	0	0	0	31	6.6	339.7	10.9
1B	26	1	16	3	1	3	50	10.7	448.2	8.9
2A	7	4	5	0	2	5	23	5.0	268.4	11.6
2B	16	2	16	0	1	0	35	7.5	466.1	13.3
3A	12	2	10	0	0	0	24	5.1	305.5	12.7
3B	17	4	19	0	0	1	41	8.8	422.7	10.3
4A	26	2	17	0	0	0	45	9.6	559.5	12.4
4B	8	2	12	0	1	8	31	6.6	484.6	15.6
5A	11	3	3	0	0	1	18	3.8	225.4	12.5
5B	8	1	12	0	1	4	26	5.6	368.7	14.1
6A	14	1	14	1	1	2	33	7.0	494.2	14.9
6B	15	1	13	1	2	3	35	7.5	509.3	14.5
7A	9	2	20	0	0	0	31	6.6	327.5	10.5
7B	8	0	27	0	0	10	45	9.6	453	10.0
Total	191	27	199	5	9	37	468	100	5672.8	12.1

bated at 65°C for 30 min. Chloroform: isoamyl alcohol (24:1) was added followed by centrifugation at 2800 rpm for 15 min. The upper phase was collected and the DNA was precipitated overnight at -20°C in 95% alcohol. After pelleting and resuspending in sterile deionized water, one-tenth of the sample was used in a test gel to verify DNA concentration. DNA was digested with eleven restriction enzymes (*Eco*R1, *Eco*RV, *Dra*I, *Xba*I, *Hin*dIII, *Bam*HI, *Pst*I, *Xho*I, *Hae*III, *Kpn*I and ScaI) and subjected to slow electrophoresis (16 h) through 0.9% agarose in TEA buffer. After ethidium bromide staining and photography, the DNA was transferred to a nylon membrane. The gels were depurinated in 0.25 N HCl for 10 min, neutralized in 0.4 N NaOH for few minutes and placed on blotting apparatus filled with 0.4 N NaOH overnight. The DNA blots were washed with 2X SSC, dried and stored at 4°C to be used for hybridization.

Probes and hybridization

Twelve candidate genes from different sources (**Table** 1) and 103 dESTs from barley (Ozuturk et al., 2002) were used as probes to detect polymorphism between the two parents. The 55 probes detecting one or more polymorphisms were subsequently analyzed for segregation among the RILs population (Table 1). The probes were denatured for 10 min and radioactively labeled by random priming using ^{32}P-dCTP. 11 µl LS (oligonucleotide mixture), 2 µl Klenow, and 3 µl ^{32}P were added to a 100 ng of DNA probe. The mixture was incubated at 37°C for 2 h then was 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 1M Na2PO4, 20% sodium dodecyl sulfate (SDS), 66.6 g/l bovine serum albumin and 10 mg/ml denatured salmon-testicle DNA. Three washing steps were conducted at 65°C using SSC buffers at 2X, 1X and 0.5X for washing steps 1, 2 and 3 respectively. 10 g per liter SDS was added to each of the washing buffers.

Genetic linkage map construction and QTL detection

The 55 probes that showed polymorphism between the parental lines were used to probe the DNA blots from the 110 RILs. For each marker, the RILs were scored as '1' or '3' for presence of the parental band of the female parent (JK) or the male parent (Cham 1), respectively, or '0' for missing data. Linkage analysis and map construction were performed by using Map Manager QTX14 (Manly and Cudmore, 1997) using the Haldane function (Haldane 1919) to convert the recombination frequencies to centiMorgans (cM). The linkage groups were constructed using the "make linkage group" command with a minimum LOD score of 3.0 followed by ripple command for each linkage group to check the final order of markers. Order information from the previously published map (Nachit et al., 2001) was also considered in some regions. Single-point analysis at significant $P \leq 0.01$ and interval mapping using Qgene program (Nelson, 1997) were employed to identify putative QTLs. Significance thresholds were established by permutation testing (Churchill and Doerge, 1995). Where trait assays were replicated, the averages over replicates were used for analysis provided that individual replicates gave no qualitative and only minor quantitative differences from the average.

RESULTS

Parental polymorphism and genetic linkage map

Of the 115 probes used, 55 (48%) detected polymerphism between the parental lines for at least one of the 11 restriction enzymes used. Fifty-three probes revealed one segregating fragment, two probes allowed 4 different loci to be mapped and 14 loci remained unlinked. In total, 162 loci (6 known genes, 37 dESTs and an additional 119 markers that were unlinked in the map developed by Nachit et al. (2001) were merged with 306 previously mapped markers (Nachit et al., 2001) to produce new map with 468 loci and an average distance between markers of 12 cM in 14 linkage groups (**Figure** 1). The distribution of markers, chromosome assignment and map coverage across the 14 durum chromosomes are summarized in **Table** 2.

Table 2. Distribution of molecular markers, assignment and centiMorgan (cM) coverage across the 14 durum A and B genome.

Chromosome	RFLPs	SSRs	AFLPs	SSPs	Genes	ESTs	Markers		cM	cM/Marker
							#	%		
1A	14	2	15	0	0	0	31	6.6	339.	10.9
1B	26	1	16	3	1	3	50	10.	448.	8.9
2A	7	4	5	0	2	5	23	5.0	268.	11.6
2B	16	2	16	0	1	0	35	7.5	466.	13.3
3A	12	2	10	0	0	0	24	5.1	305.	12.7
3B	17	4	19	0	0	1	41	8.8	422.	10.3
4A	26	2	17	0	0	0	45	9.6	559.	12.4
4B	8	2	12	0	1	8	31	6.6	484.	15.6
5A	11	3	3	0	0	1	18	3.8	225.	12.5
5B	8	1	12	0	1	4	26	5.6	368.	14.1
6A	14	1	14	1	1	2	33	7.0	494.	14.9
6B	15	1	13	1	2	3	35	7.5	509.	14.5
7A	9	2	20	0	0	0	31	6.6	327.	10.5
7B	8	0	27	0	0	10	45	9.6	453	10.0
Total	191	27	199	5	9	37	468	10	5672	12.1

Detection of QTLs

A total of 335 QTLs with a P value ≥ 0.01 were identified for the drought-related traits evaluated in different environments (Figure 1). Multiple QTLs were found for all traits except osmotic potential and osmotic potential at full turgor, where a single QTL was identified for each of them on chromosome 2A. Sixty-three QTL were identified for canopy temperature depression, 43 for chlorophyll content, 7 for fluorescence indices, 29 for carbon isotope discrimination, 7 for osmotic adjustment, 62 for photosynthetically active radiation, 56 for quantum yield, 3 for relative water content, 32 for transpiration and 31 for water index. Many of the QTL for different traits were overlapping indicating that either there are closely linked genes or the same gene is affecting different traits. Over all environments, the number of QTLs identified for each trait varied from 1 to 34 with the phenotypic variation (R^2) ranging from 3.4 to 55%.

Candidate genes co-segregating with drought traits

Comparison of genome locations for QTLs for drought tolerance and candidate genes showed coincidences on chromosomes 1B, 2A, 3B, 4B, 5A, 5B, 6A, 6B and 7B (Figure 1 and Table 3). For the IR environment, 3 genes and 6 dESTs were found to be associated with QTLs for canopy temperature depression, chlorophyll content, and photosynthesis active radiation. In the RF environment, QTL for chlorophyll content, fluorescence indices, carbon isotope discrimination, osmotic potential, photosynthesis active radiation, quantum yield, transpiration and water index were associated with 4 candidate genes and 15 dESTs. Three candidate genes and 7 dESTs were associated with QTL for canopy temperature depression and and quantum yield under LP (Figure 1 and Table 3).

Correlation between traits

Some genomic regions were found where QTLs for different traits overlapped. For example, QTL for canopy temperature depression, photosynthetically active radiation, water index and quantum yield were often mapped to the same chromosomal locations (PaggMcag1, PaggMctg7, PaggMcgt12, PaagMctg9, PaagMcgc9) as were the QTL for transpiration, carbon isotope discrimination, photosynthetically active radiation, water index and quantum yield (PacgMcag8, PaggMcag8, PaagMcag5). QTLs for canopy temperature depression and chlorophyll content were frequently located in the same regions (PacgMcgc4, PaccMcga11, PacgMcag8, and PaccMcga12). Also QTL for carbon isotope discrimination and transpiration were mapped to the same locus (gwm389) (Figure 1).

DISCUSSION

Genetic map

The development of genetic linkage map is a first step towards the detection of factors controlling the expression of important traits. The new map consists of 468 loci covering 5672.8 cM, whereas the previous maps developed by Blanco et al. (1998) for the cross durum X *Triticum dicoccoides* and by Nachit et al. (2001) consisted of 259 loci covering 1352 cM and 306 loci covering 3598 cM, respectively. In this mapping population the marker distribution was relatively adequate and few clusters of tightly linked loci were found. For the mapped

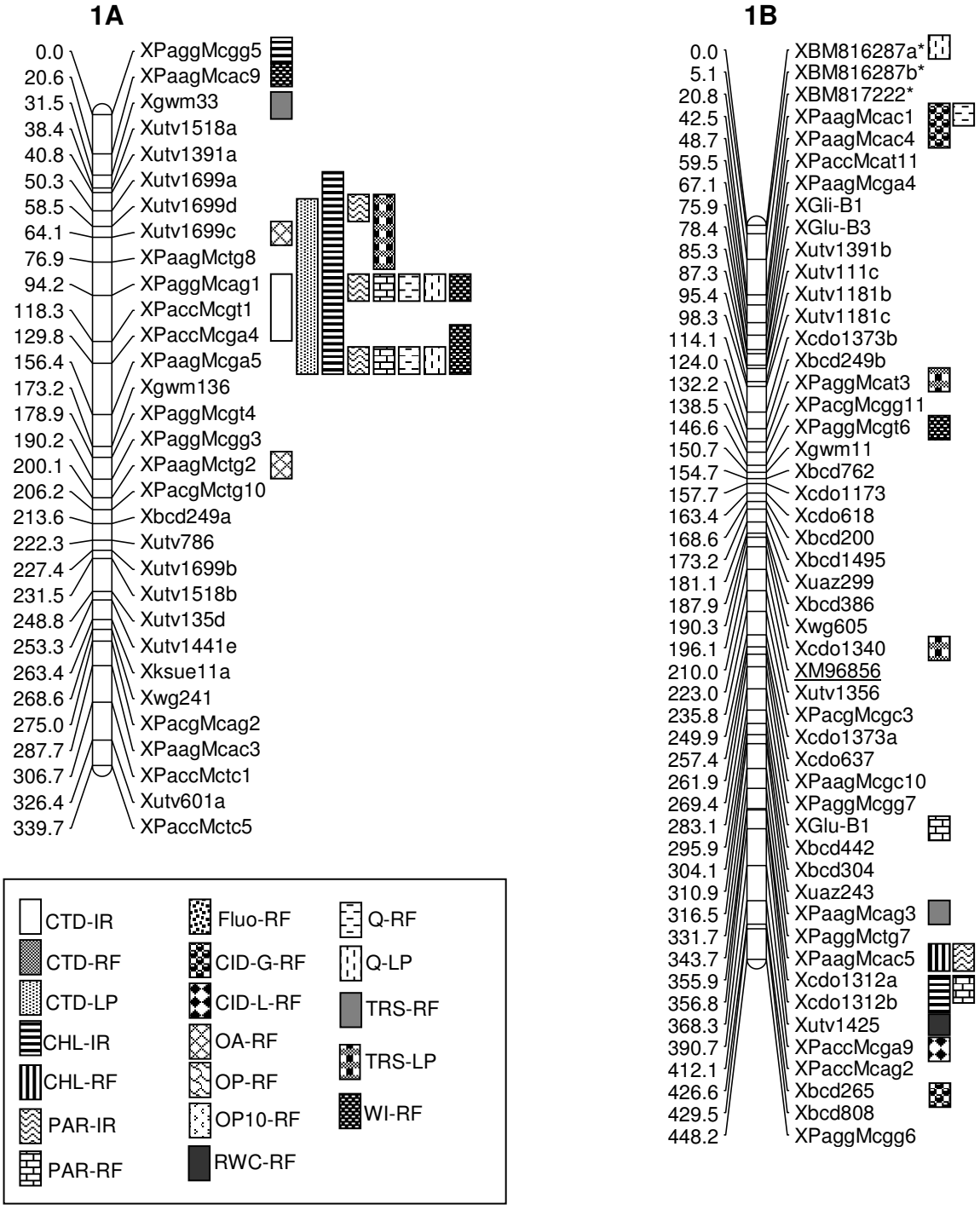

Figure 1. Linkage map of durum wheat (*T. turgidum* L. var. durum) showing positions of QTL influencing canopy temperature depression (CTD), chlorophyll content (CHL), fluorescence (Fluo), carbon isotope discrimination (CID), osmotic adjustment (OA), osmotic potential (OP), osmotic potential at full turgor (OP10), Photosynthesis active radiation (PAR), quantum (Q), relative water content (RWC), transpiration (TRS) and water index (WI). IR: irrigation, RF: rainfed, LP: late planting. Markers with an * are dESTs and genes are underlined.

ESTs, the results showed clearly that on the genome more ESTs were mapped than on the A genome suggesting that the B genome was more polymorphic than the A genome. The map orientation of this map was based on the previous linkage map developed by Nachit et al. (2001) and there was generally good agreement for marker order. However, five gaps on chromosomes 1A, 2B, 3A, 6A and 7B, 4 gaps on chromosomes 1B, 2A, 3B,

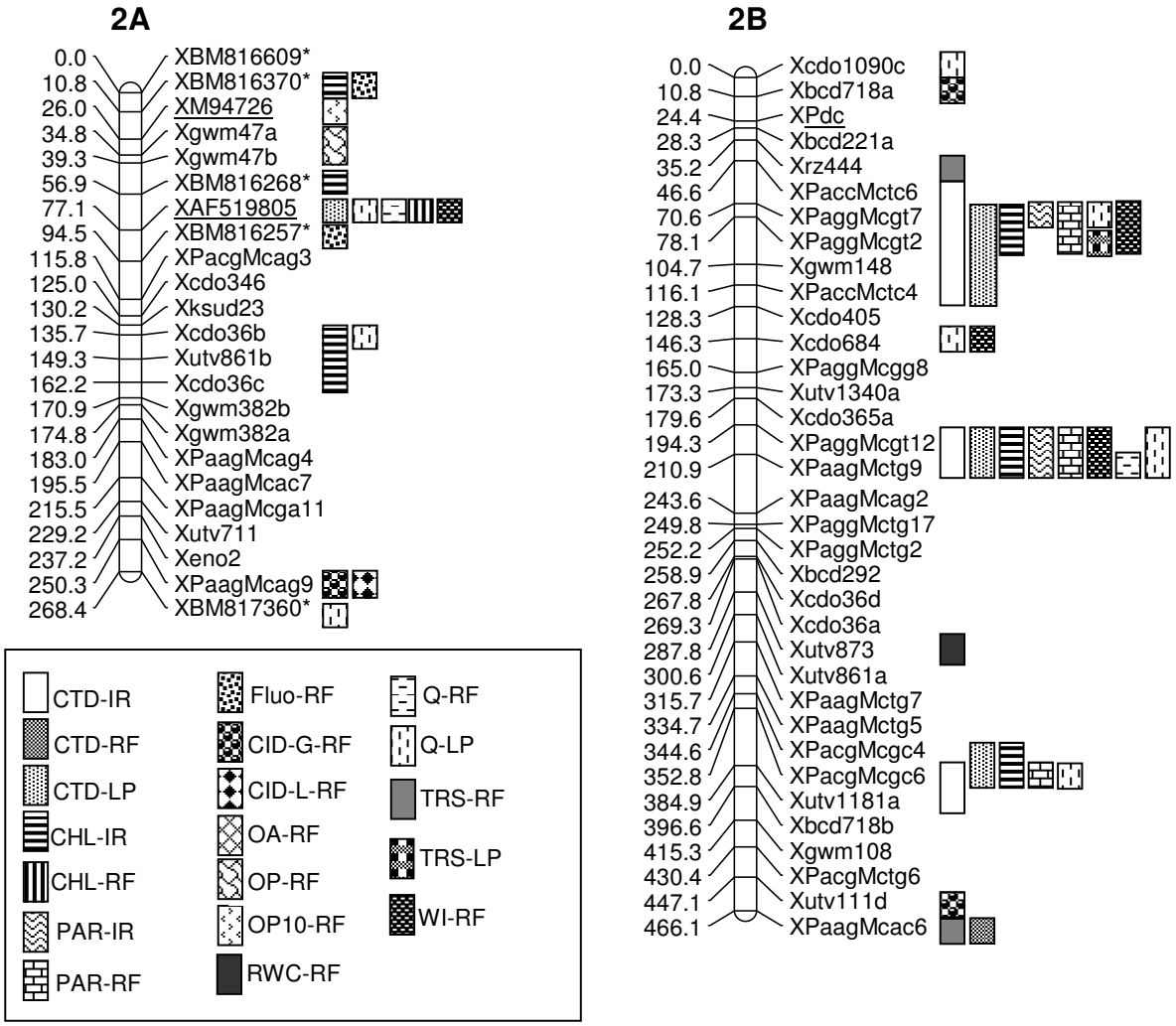

Figure 1. contd.

5B, and 2 gaps on 3A were eliminated or reduced. The chromosome with the most markers is chromosome 1B (50 markers), followed by 4A (46 markers), 7B (45 markers) and 3B (41 markers). The chromosomes with the fewest markers are 5A (19 markers), 2A (23 markers) and 3A (24 markers).

Correlations between traits

Correlations between traits can be interpreted according to their physiological effects to determine their relevance to plant improvement under drought stress. Preliminary information can be obtained from a correlative approach conducted on a large population and from a colocation (QTL-QTL, candidate gene-QTL) analysis within the same species or within related species.

In this work, many genomic regions were identified with significant effects on more than one trait. QTLs for photosynthetically active radiation, canopy temperature depression, chlorophyll content, transpiration and carbon isotope discrimination were often mapped to the same region. The correlation between these traits have been reported in durum wheat (Royo et al., 2002; Villegas et al., 2000; Merah et al., 2001; Tambussi et al., 2002.), bread wheat (Richards and Condon, 1993) and in barley (Acevedo, 1993). Physiologically, canopy temperature and carbon isotope discrimination are indicators of the photosynthetic/transpirative activity of the crop in a particular environment. Thus, canopy temperature indirectly reflects the instantaneous transpiration at the whole crop level (Reynolds et al., 1994). Carbon isotope discrimination integrates the transpiration efficiency (that is, the ratio of net photosynthesis to water transpired) over the period during which the seed dry matter was accumulated (Farquhar and Richards, 1984; Condon et al., 1990). Higher carbon isotope discrimination is sustained by a higher ratio of intercellular to external partial pressure of CO_2 during photosynthesis due a higher stomatal conductance (Farquhar and Richards, 1984; Farquhar et al., 1989). A decrease in canopy temperature is associated with more water transpired by the canopy (Blum, 1988) and these traits are related (Araus et al., 1993).

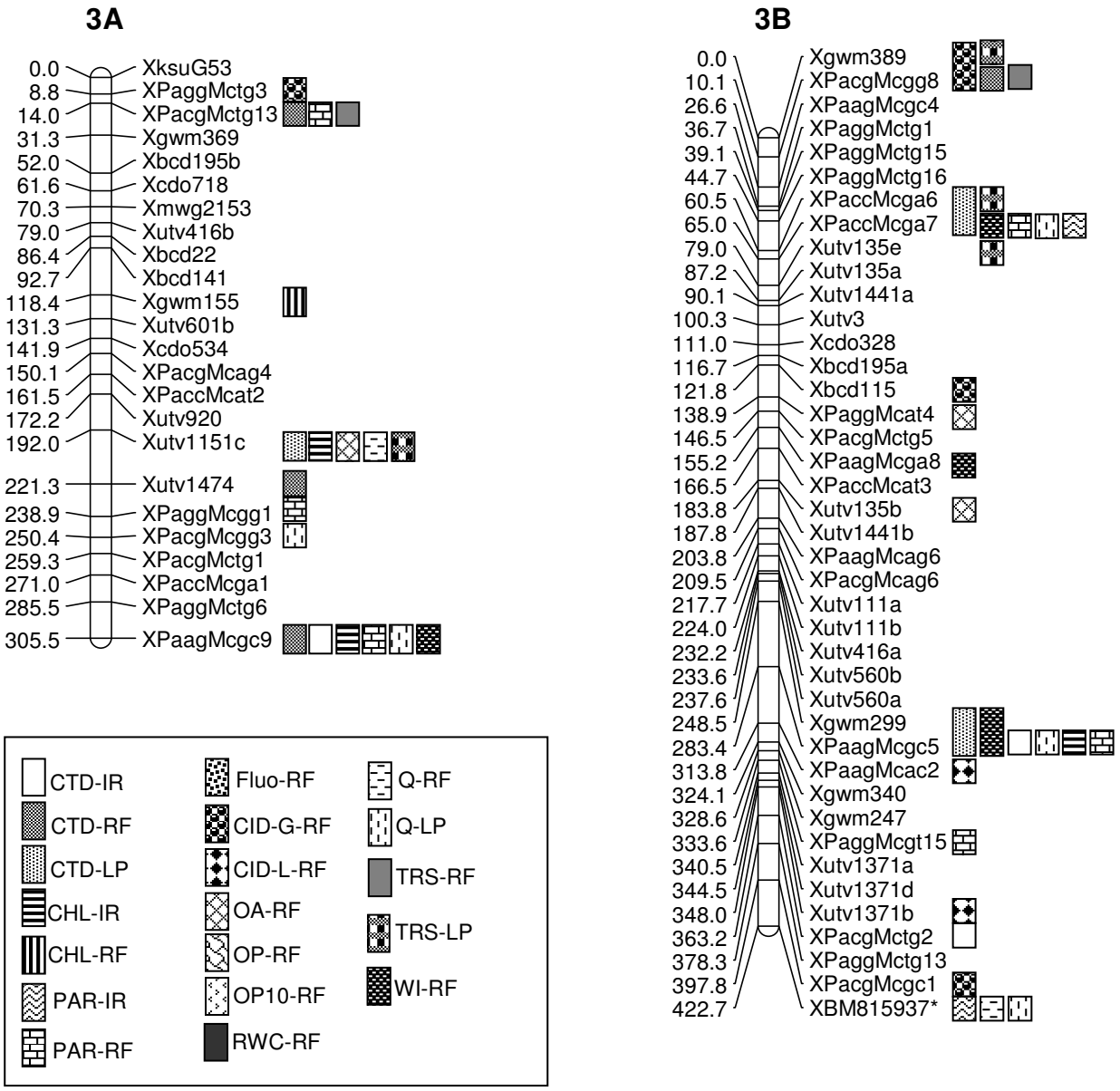

Figure 1. contd.

Total chlorophyll content is an indication of the potential photosynthetic capacity and these two traits are correlated (Araus et al., 1997). Therefore, the co-localization of QTL for these traits is most likely due to pleiotropic effects of the same gene(s). The distinction between linkage and pleiotropy is impor-tant for breeding purposes as well as for scientific reasons. However, without fine resolution mapping or molecular cloning of QTLs, such distinction would be difficult and at best one can make inferences based on morphological and/or physiological relationships between traits under consideration. The ability to dissect the gene-tic control of these traits also allows one to determine which assay is most efficient for detecting superior alleles within and across loci for this population.

Candidate genes and differently expressed sequences co segregating with drought related traits

The co-localization of specific genes with QTLs can be an efficient approach to identifying the genes controlling drought tolerance or traits related to drought response. The primary goal of this research was to identify associations between QTL for drought tolerance and candidate genes. Six candidate genes and 19 different osmotic potential at full turgor, transpiration, canopy temperature depression, water index, photosynthetic active radiation and grain carbon isotope discrimination (Figure 1 and

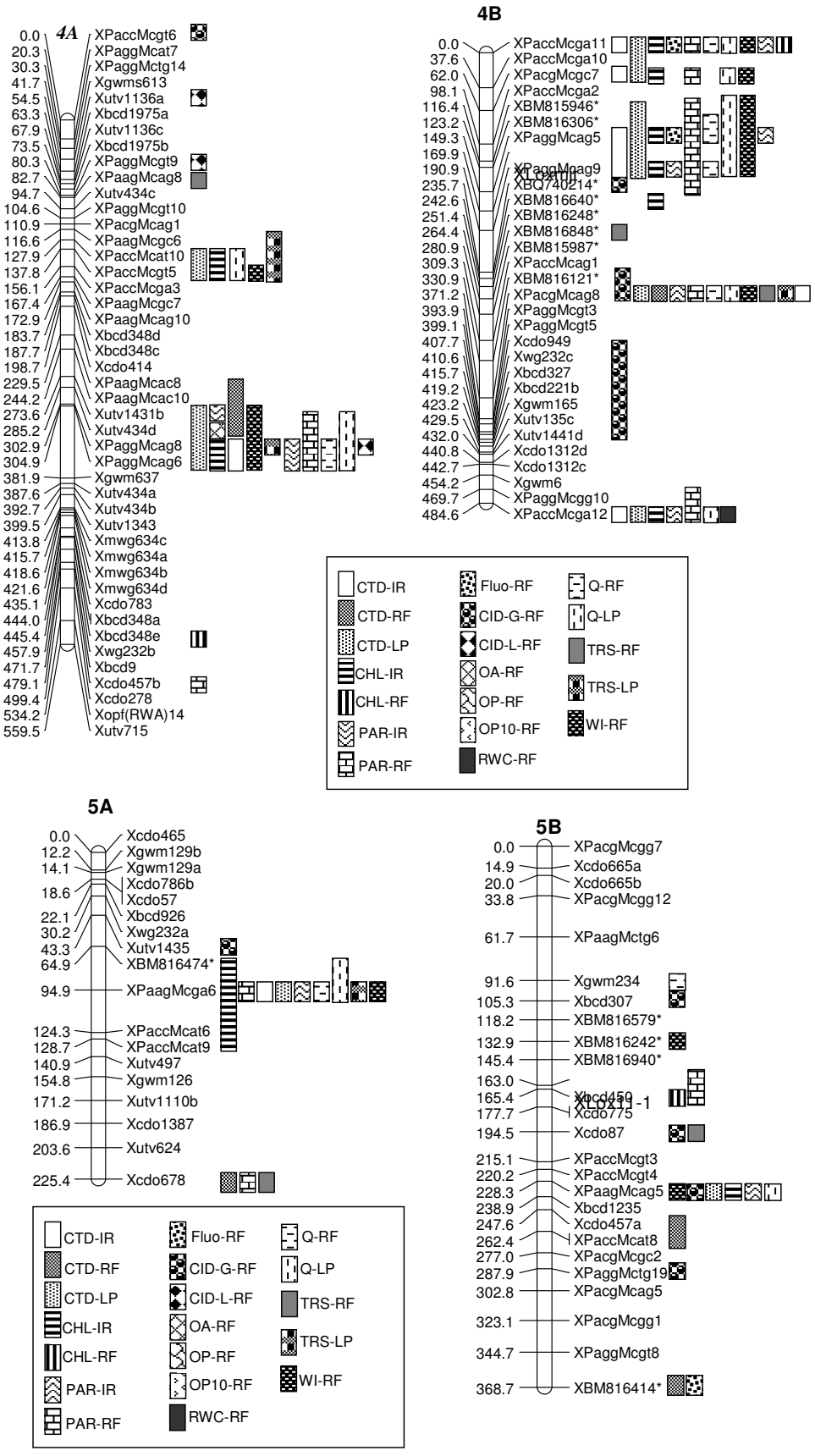

Figure 1. contd.

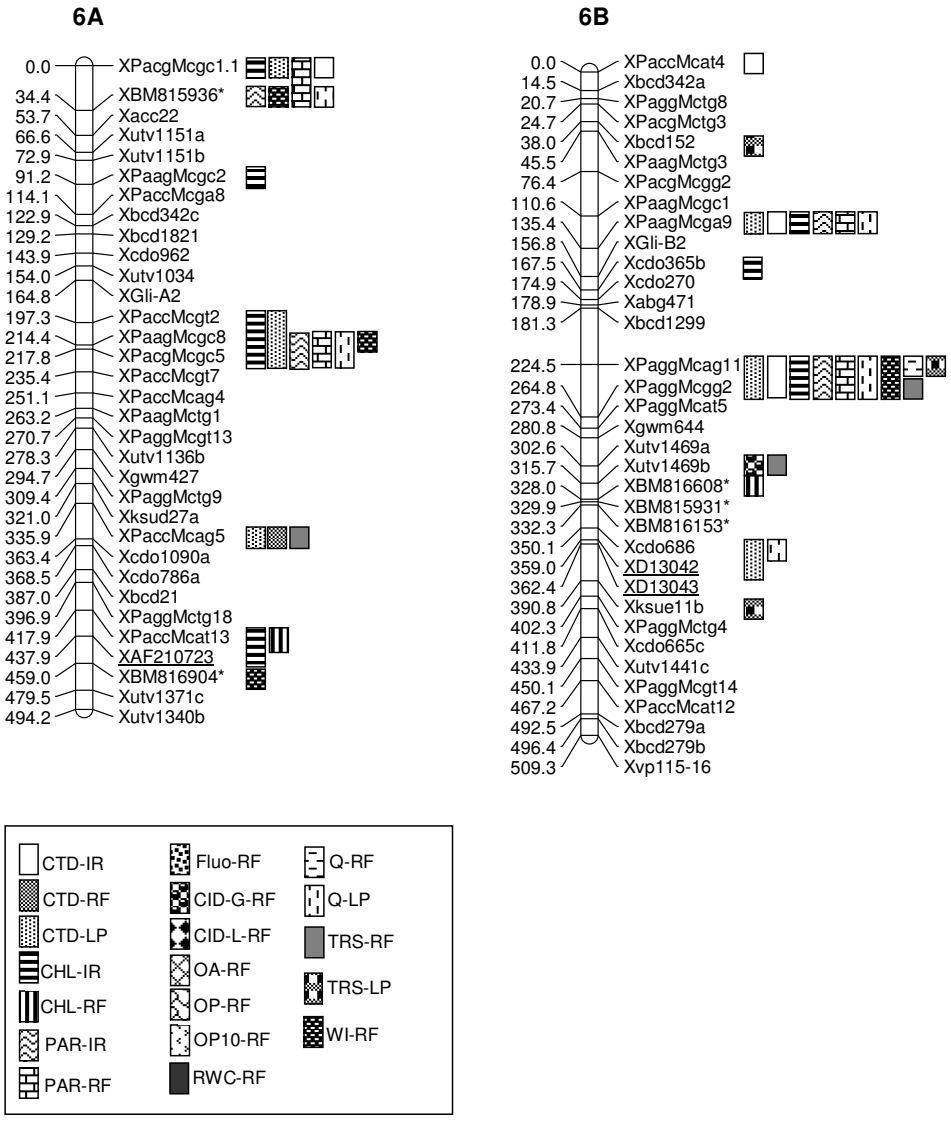

Figure 1. contd.

Table 3). These traits are components of drought tolerance and have been proposed as selection criteria for drought in durum wheat (Nachit, 2000; Araus et al., 1997; Rekika et al., 1998).

Candidate genes

The genes M94726, AF519805 and D13042, coding for protein kinase, co-segregated with QTL for osmotic potential at full turgor and QTL for canopy temperature depression, quantum yield, chlorophyll content and water index. M94726 and AF519805 were originally cloned from dormant seed embryos of wheat and their transcripts accumulate in ABA-treated embryos and in severely dehydrated or cold-treated seedlings (Anderberg and Walker-Simmons, 1992). While the homolog of the D13042 gene (responsive to desiccation) has been cloned from *Arabidopsis thaliana*, is not induced by ABA, and contains signal peptides that function in protein secretion under dehydration (Koizumi et al., 1993). ABA is a growth regulator, which accumulates when plant tissues are dehydrated. In view of the variety of regulatory systems involved in the expression of drought-inducible genes, there appear to be several signal-transduction pathways. Because M94726 and AF519805 are ABA-responsive protein kinases, they are potential candidates for an intermediate in ABA signaling pathways. However, there several genes that are responsive to desiccation stress that do not respond to ABA (Guerrero et al., 1990), indicating the existence of ABA-independent signal transduction. Water deficit induces stomatal closure in leaves, which reduces further water loss, and inhibits leaf and stem growth and photosynthesis.

The loci Loxmjt and Lox11-1 (only PAR) genes coding

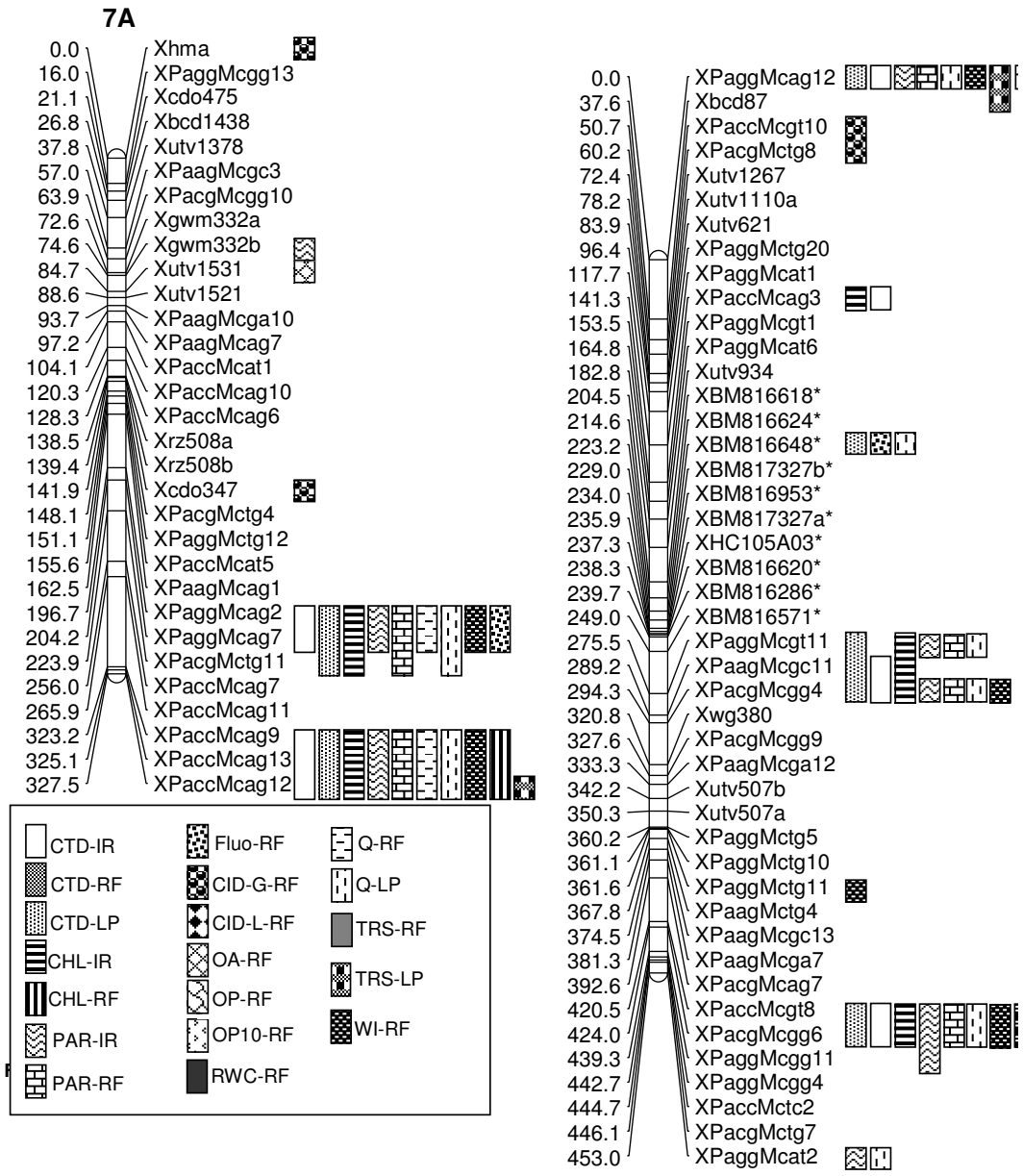

Figure 1. contd.

for lipoxygenase, co-segregated with QTL for canopy temperature depression, photosynthetically active radiation, quantum yield water index on chromosome 4B and with a QTL for photosynthetic active radiation on chromosome 5B respectively. Lipoxygenase is a non-heme iron-containing enzyme, which catalyzes the hydroperoxidation of fatty acids and is modulated in plants by water deficit (Bell and Mullet, 1991). Despite its wide distribution in the plants, the physiological role of this gene has only partly been elucidated. Lipoxygenase has been proposed to play a role in senescence, pathogen, and wound responses and has also been implicated in the biosynthesis of ABA (Vick and Zimmerman, 1987). Bell and Mullet (1991) reported that exposure of soybean seedlings to drought stress results in a rapid change in lipoxygenase levels relative to the well watered plants. This rapid change may be related to increased synthesis of ABA in the stressed plants. The possible involvement of lipoxygenase in ABA biosynthesis has been previously suggested based on work by Firn and Friend (1972), who showed that this gene could convert violaxanthin to xanthoxin in presence of linoleate. Xanthoxin is a precursor of ABA, and conversion of an oxygenated carotenoid to xanthoxin may be the rate-determining step in ABA biosynthesis in stressed plants (Sindhu and Walton, 1987). Ingram and Bartels (1996) have grouped the lipoxygenase gene, isolated from soybean, among genes that are upregulated by drought. Also it has been report-

Table 3. Differentially expressed sequence tags and candidate genes co-segregating with drought related traits in different environments.

Locus	Gene product	Chr	Associated trait(s)	Environment
BM816287a	Putative protein, Arabidopsis	1B	Quantum yield	Late planting
BM816370	Alcohol dehydrogenase	2A	Chlorophyll content	Irrigation
			Fluorescence indices	Rainfed
M94726	Protein kinase	2A	Osmotic potential at full turgor	Rainfed
BM816268	Ubiquitin-protein ligase	2A	Chlorophyll content	Irrigation
AF519805	Protein kinase	2A	Canopy temperature depression	Late planting
			Quantum yield	Late planting , Rainfed
			Chlorophyll content	Rainfed
			Water index	Rainfed
BM816257	Actin depolymerizing factor 4	2A	Fluorescence indices	Rainfed
BM817360	Sugar transporter	2A	Quantum yield	Late planting
BM815937	Blue copper-binding protein	3B	Photosynthetic active radiation	Irrigation
			Quantum yield	Late planting
			Quantum yield	Rainfed
BM815946	Unknown	4B	Canopy temperature depression	Late planting
			Photosynthetic active radiation	Rainfed
			Quantum yield	Late planting
			Water index	Rainfed
BM816306	Oxalate oxidase	4B	Canopy temperature depression	Late planting
			Photosynthetic active radiation	Rainfed
			Quantum yield	Late planting
			Quantum yield	Rainfed
			Water index	Rainfed
Loxmjt	Lipoxygenase	4B	Photosynthetic active radiation	Rainfed
			Quantum yield	Late planting
			Water index	Rainfed
BQ740214	unknown	4B	Grain carbon isotope discrimination	Rainfed
			Photosynthetic active radiation	Rainfed
BM816640	Phosphoprotein phosphatase	4B	Chlorophyll content	Irrigation
BM816848	Hypothetical protein	4B	Transpiration	Rainfed
BM816121	Stearoyl-CoA desaturase	4B	Grain carbon isotope discrimination	Rainfed
BM816474	Cathepsin B	5A	Chlorophyll content	Irrigation
BM816242	Glutathione S-transferase	5B	Water index	Rainfed
Lox11-1	Lipoxygenase	5B	Photosynthetic active radiation	Rainfed
BM816414	Early flowering protein 1	5B	Canopy temperature depression	Rainfed
			Fluorescence indices	Rainfed
BM815936	ORF107a, Arabidopsis	6A	Photosynthetic active radiation	Irrigation
			Photosynthetic active radiation	Rainfed
			Water index	Rainfed
			Quantum yield	Late planting
AF210723	Fructan fructosyltransferase	6A	Chlorophyll content	Irrigation
BM816904	Serine/arginine-rich protein	6A	Water index	Rainfed
BM816608	Glutathione oxidase	6B	Chlorophyll content	Rainfed
D13042	Protein kinase	6B	Canopy temperature depression	Late planting
BM816648	Arginine decarboxylase 2	7B	Canopy temperature depression	Late planting
			Fluorescence indices	Rainfed
			Quantum yield	Late planting

ed that lipoxygenase revealed an N-terminal extension that could be a signal for chloroplast targeting (Fuks and Schnell, 1997). A gene encoding a chloroplast-targeted lipoxygenase in tomato leaves has been cloned and characterized (Heitz et al., 1997). Although the physiological function of lipoxygenase gene in plants is not well defined, the association of this gene or linked genes with components of drought tolerance suggests a role for this gene in drought tolerance in durum wheat. The locus AF210723 is homologous to a gene isolated and characterized in barley and coding for fructan fructosyltransferase (FFT) (Sprenger et al., 1995), is associated with a QTL for chlorophyll content on chromosome 6A. Fructans are a class of highly water-soluble polysaccharides consisting of linear or branched fructose chains attached to sucrose (Sprenger et al., 1995). They also represent a major nonstructural carbohydrate in many plant species including wheat and barley (Lewis, 1993; Edelman and Jefford, 1968). Fructans accumulate in the vacuole and have a role in osmoregulation (Bieleski, 1993) and an important function in the temporary storage and partitioning of assimilates (Pollock and Cairns, 1991; Hendry, 1993). Fructans also may play a role in resistance to drought and cold stress (Pontis and Del Campillo, 1985; Pollock, 1986; Hendry, 1993) as supported by the finding that tobacco, a species normally incapable of forming fructans, shows improved drought resistance upon transformation with gene encoding a bacterial fructan-forming enzyme (Pilon-Smits et al., 1995). Pilon-Smits et al., (1995) suggested that the amount of fructan accumulated in the tobacco leaves seemed too low to have an osmotic effect. They also suggested that fructan might protect membranes or other cellular components against the adverse effects of drought or its biosynthesis might influence the process of cell wall hardening, which is the one of the first reactions to water stress (Chazen and Neumann, 1994). The association between the FFT gene and chlorophyll content in this study suggests another role of this gene in the photosynthesis of durum wheat. Lu et al. (2002) reported that photosynthesis and carbohydrate metabolisms are linked and highly cell specific in barley. It is also well known that the epidermis is effectively sugar-free, and the mesophyll and parenchymatous bundle sheath have different patterns of starch and fructan accumulation in response to drought stress (Williams et al., 1989; Koroleva et al., 2001). More over, the expression of the barley fructan gene is modulated by prolonged light treatment (Giuliano et al,. 1988; Koroleva et al., 2000). This supports the finding of Lu et al. (2002) who reported that the concentration of fructan in barley plants decreased gradually in continuous darkness and increased in light. Though FFT co-segregates with the QTL for chlorophyll content the possible biochemical link between this gene and the trait is not clear. However, linkage of the locus coding for FFT suggests that it might have a role in drought tolerance in durum through reducing chlorophyll loss.

Differentially expressed sequences

The location of differentially expressed sequence tags at QTLs involved in drought tolerance could give some information about their role. The dESTs that have unknown function and co-segregated with QTL for drought components include BM816287a, BM815946, BM816848, BM815936 and BQ740214 (Table 3). The association between these dESTs and QTL for drought tolerance suggests a possible role in drought tolerance in durum wheat; however, more research is needed to identify functions for those dESTs and to validate their role.

The loci BM816640, BM816370, BM816257, BM817360, BM815937, BM816306, BM816242, BM816474, BM816648, BM816268, BM816121, BM816414, BM816608 and BM816904 coding respecttively for phosphoprotein phosphatase, alcohol dehydrogenase, actin depolymerizing factor, sugar transporter, blue copper-binding protein, oxalate oxidase, glutathione S-transferase, cathepsin B, arginine decarboxylase, ubuquitin-protein ligase, stearoyl-CoA desaturase, early flowering protein, glutathione oxidase and serine/arginine-rich protein, (Ozturk et al., 2002) also co-segregated with several QTLs for components of drought tolerance (Table 3). The role of these ESTs in drought tolerance has not yet been established. However, studies report a role for some of these genes in biotic and abiotic stresses. For example, phosphoprotein phosphatase is an osmoprotectant and regulates salinity, osmotic tolerance, and plant growth in Arabidopsis (Espinosa-Ruiz et al., 1999). Stearoyl-CoA desaturase is a soluble enzyme that catalyzes the insertion of double bond into saturated fatty acids in plants and is considered a key enzyme in fatty acid desaturation (Nagai and Bloch, 1968). Since membrane fatty acid composition plays a key role in regulating membrane fluidity and cell signaling, this gene could have a role in the protection of membranes or in membrane fluidity during drought stress. The expression of this gene appears to be related to various environmental stress responses (Im et al., 2001).

The locus BM816370, coding for alcohol dehydrogenase, co-segregated with QTL for chlorophyll content and fluorescence indices on chromosome 2 A. It is well known that photosynthetic systems in higher plants are sensitive to drought and other abiotic stresses (Falk et al., 1996). The effect of drought stress on photosynthesis has been a subject of controversy among plant physiologists for many years, and conflicting results have been reported depending on the plant material, and the experimental procedures used for investigation (Cornic and Massacci, 1996). It has not been well established how the chloroplasts are damaged by drought stress. Chlorophyll fluorescence is one of the physiological parameters that have been shown to correlate with drought stress and other abiotic stress (Belkhodja et al., 1994). Alcohol dehydrogenase is induced by low oxygen stress

in many plants and known to play an important role to survive adverse conditions (Kennedy et al., 1992; Ricard et al., 1994; Drew, 1997; Kato-Noguchi, 1999). Drought and temperature stresses induced the expression of the alcohol dehydrogenase gene in *Arabidopsis* (Jarillo et al., 1993; Dolferus et al., 1997; Conley et al., 1999). It is also reported that this gene is induced by abscisic acid in *Arabidopsis* (Bruxelles et al., 1996) and its activity is increased under osmotic stress in maize (Kato-Noguchi, 2000). The co-segregation of the alcohol dehydrogenase gene with QTL for chlorophyll content and fluorescence indices does not indicate a function for this gene in drought tolerance but it may have a role in reducing chlorophyll loss and maintaining photosynthesis.

The locus BM816257, coding for actin depolymerizing factor (ADF), co-segregated with fluorescence indices on chromosome 2 A. The ADF is part of the ADF/cofilin group, a family of small proteins (15 – 22 kD) that includes cofilin, destrin, depactin, and actophorin (Staiger et al., 1997; Lappaleinen et al., 1998). The members of this family are stimulus-responsive modulators of the cell actin cytoskeleton dynamics. They show actin monomer binding, actin-filament binding/severing, and nucleotide / monomer dissociation-inhibiting activities *in vitro* (Lappalainen et al., 1997; McGough and Chiu, 1999). Using Arabidopsis ADF1, Carlier et al. (1997) have suggested that one of the main functions of ADF is to increase the turnover rate of actin filaments. Several cellular processes are associated with the reorganization of the actin cytoskeleton in plants. These include cell division and differentiation, stomatal movement, gravitro-pic tip growth, light induced plastid migration, wound repair, response to pathogen attack, pollen development, nuclear migration, cytoplasmic streaming, secretion, cell wall biosynthesis, and transmembrane signaling (Aon et al., 1999). Actin filaments are tightly linked to the plasma membrane and believed to be involved in signal transduction events in plants (Aon et al., 1999). Disruption or reorganization of the cytoskeleton could thus impair or modify the activity of signaling molecules associated with cytoskeletal elements. Based on the above information and on the fact that ADF has shown to be an indicator for drought response in rice (Leung, 2001) and for cold stress in wheat (Ouellet et al., 2001), the association between ADF and QTL for fluorescence indices suggests that important changes in the actin cytoskeletal architecture may occur during drought stress, and that these modifications may be related to fluorescence indices in durum wheat under moderate drought (RF) conditions.

The locus BM817360, coding for a sugar transporter, is associated with a QTL for quantum yield on chromosome 2 A. Quantum yield is a measure of photosynthetic efficiency expressed in moles of photons absorbed per mole of CO_2 fixed or O_2 evolved and sugar transport is a fundamental process for the allocation of assimilates. It is reported that under light conditions, the sugar transporter gene transcription is rapidly induced (Matsukura et al., 2000). Light may affect induction directly through photoreceptors and indirectly through carbohydrate accumulation arising from photosynthesis. Some sugar transporters have been cloned from different species, e.g. potato, tomato, *Arabidopsis*, *Plantago* and rice (Gahrtz et al., 1994; Sauer and Stolz, 1994; Hirose et al., 1997; Kuhn et al., 1997). Although significant advances have been achieved in cloning plant sugar transporters, little is known about the regulation and function of most carrier systems that contribute to assimilate partitioning in the plant. This study shows a possible role of sugar transporter in drought tolerance through an association between this gene and quantum yield under severe drought stress (LP).

The loci BM815937, BM816306, and BM816242 coding receptively for blue-copper-binding protein, oxalate oxidase and glutathion S-transferase co-segregated with QTLs for photosynthetically active radiation, canopy temperature depression, quantum yield and water index. A gene coding for blue-copper-binding 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). Six oxalate oxidase genes, known to be expressed in cell walls of cereal embryos, have been isolated and characterized in barley and two of those are salt-responsive proteins (Hurkman et al., 1991; Lane et al., 1993; Lane, 1994; Hurkman et al., 1994; Dumas et al., 1993; Wei et al., 1998). A gene coding for glutathion S-transferase has been used to transform tobacco (Roxas et al., 2000) and *Arabidopsis* (Ezaki et al., 2000). The transformed plants obtained form these studies showed sustained growth under cold and salinity stress and resistance against aluminum toxicity and oxidative stress. It has been reported that exposure of plants to various environmental perturbations, including drought, intense light, temperature stress, the presence of metal toxicity (e.g Aluminum), can lead to the generation of activated oxygen species (AOS) (Datta, 2002). These AOS cause extensive cellular damage, due to oxidative stress, and inhibition of photosynthesis (Allen, 1997). Plants have evolved systems to combat this oxidative stress with a battery of gene products that aid in reducing the AOS that damage membranes. Enzymes such as oxalate oxidase and glutathion S-transferase in addition to the gene coding for blue copper binding protein are involved in such protective processes (Zhou et al., 1998; Datta, 2002; Halliwell and Gutteridge, 1984; Kampfenekel et al., 1995).

The locus BM816474, coding for cathepsin B, co-segregated with a QTL for chlorophyll content on chromosome 5 A. Cathepsin B is an ancient family of eukaryotic cysteine proteases (Vincent et al., 2000). The cathepsin B proteases were originally identified in mammalian systems as lysosomal, hydrolytic enzymes. Because they can degrade a wide range of peptide/protein substrates,

transferase in addition to the gene coding for blue copper binding protein are involved in such protective processes (Zhou et al., 1998; Datta, 2002; Halliwell and Gutteridge, 1984; Kampfenekel et al., 1995). The locus BM816474, coding for cathepsin B, co-segregated with a QTL for chlorophyll content on chromo-some 5 A. Cathepsin B is an ancient family of eukaryotic cysteine proteases (Vincent et al., 2000). The cathepsin B proteases were originally identified in mammalian sys-tems as lysosomal, hydrolytic enzymes. Because they can degrade a wide range of peptide/protein substrates, a role in cellular protein turnover has been indicated (re-viewed in Bond and Butler, 1987). Most cathepsins are glycoproteins, showing proteolytic activity against a wide range of small peptides and large protein substrates and conesquently are thought to play an important role in cellular protein turnover (Bond and Butler, 1987). There have been few reports of cathepsin B-like sequences in plants (Ward et al., 1997). A gibberellin-responsive mRNA was isolated from wheat and subsequently shown to be expressed in the scutellar parenchyma of embryos and in the aleurone layer, but not in roots (Cejudo et al., 1992). In *Nicotiana rustica,* another cathepsin B-like pro-tein coded by a wounding-responsive mRNA was isolated from roots and expressed in most plant organs (Lidgett et al., 1995). The results obtained in this study indicate a possible linkage between this gene and maintenance of chlorophyll content under rainfed conditions. Because this gene degrades a wide range of peptide/protein sub-strates it might be involved in the degradation of some proteins that cause cell damage in response to drought stress.

The locus BM816648, coding for arginine decarboxy-lase, co-segregated with QTL for canopy temperature depression, fluorescence indices and quantum yield on chromosome 7B. Arginine decarboxylase is a key en-zyme in polyamine biosynthesis. Capell et al. (1998) over-expressed an oat arginine decarboxylase gene in rice and the plants showed improved drought tolerance in terms of chlorophyll loss. Therefore, the association of this gene with photosynthesis-related traits in durum wheat strongly suggests a role in drought tolerance by reducing chlorophyll loss, hence enhancing the photo-synthesis under RF and LP conditions.

Understanding the roles of some of these candidate genes and differentially expressed sequences could lead to identification of novel drought responsive mechanisms in durum wheat. However, the observation of colocation between a QTL and a candidate gene does not provide definitive evidence for the role of the genes in trait varia-tion. Fine mapping and analysis of gene polymorphism in coding and regulatory regions are required. Comparative mapping is another indirect but valuable method that can be used to validate the QTL association taking the advan-tage of possible co-location of the same QTL/candidate gene couples in different species of the same family.

With the development of molecular markers for many quantitative trait loci (QTL) regulating specific drought responses. By comparing the coincidence of such QTL with candidate genes for drought tolerance it is possible to test more precisely whether a particular constitutive or adaptive response to drought stress would be useful in the improvement of crops for drought tolerance.

REFERENCES

Acevedo E (1993). Potential for carbon isotope discrimination as a selection criterion in barley breeding. In: Ehleringer JR, Hall AE, Farquhar GD (eds) stable isotope and plant carbon-water realtions. Academic Press, New York. pp 399-417.

Allen RD (1997). Use of transgenic plants to study antioxidant defenses. Free Radic. Biol. Med. 23: 473-479.

Anderberg RJ, Walker-Simmons MK (1992). Isolation of a wheat cDNA clone for an abscisic acid-inducible transcript with homology to protein kinases Proc. Natl. Acad. Sci. U.S.A. 89 (21), 10183-10187.

Aon MA, Cortassa S, Gomez Casati DF, Iglesias AA (1999). Effects of stress on cellular infrastructure and metabolic organization in plant cells. Int Rev Cytol. 194:239–273

Araus JL, Amaro T, Zuhair Y, Nachit MM (1997). Effect of leaf structure and water status on carbon isotope discrimination in fieldgrown durum wheat. Plant Cell Environ. 20:1484-1494.

Araus JL, Reynolds MP, Acevedo E (1993). Leaf posture, grain yield, growth, leaf structure and carbon isotope discrimination in wheat. Crop Sci. 33: 1273-1279.

Barrs HD, Weatherley PE (1968). A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust. J. Biol. Sci. 15: 413-428.

Belkhodja R, Morales-Fermin, Abadia-Anunciacion, Gomez-Aparisi J, Abadia-Javier (1994). hlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (Hordeum vulgare L.). Plant Physiol. 104: 667-673.

Bell E, Mullet JE (1991). Lipoxygenase gene expression is modulated in plants by water deficit, wonding, and methyl jasmonate. Mol. Gen. Genet. 230: 456-462.

Bieleski RL (1993). Fructan hydrolysis drives peta1 expansion in the ephemeral daylily flower. Plant Physiol. 103:213-219.

Blanco A, Bellomo P, Cenci A, De Giovanni C, D'Ovidio R, Iacono E, Laddomada B, Pagnotta MA, Porceddu E, Sciancalepore A, Simeone R, Tanzarella OA (1998). A genetic linkage map of durum wheat. Theo Appl Genet 97:721-728.

Blum A, Munns R, Passioura JB, Turner NC (1996). Genetically engineered plants resistant to soil drying and salt stress: how to interpret osmotic relations. Plant. Physiol. 110: 1051-1053.

Bond J S, Butler PE (1987). Intracellular proteases. Annu. Rev. Biochem. 56: 333-364.

Bruxelles GL, Peacock WJ, Dennis ES, Dolferus R (1996). Abscisic acid induces the alcohol dehydrogenase gene in Arabidopsis. Plant Physiol. 111: 381-391.

Capell T, Escobar C, Liu H, Burtin D, Lepri O, Christou P (1998). Overexpression of the oat arginine decarboxylase cDNA in transgenic rice Oryza sati6a L.) affects normal development patterns *in vitro* and results in putrescine accumulation in transgenic plants. Theor. Appl. Genet. 97: 246-254.

Carlier MF, Laurent V, Santolini J, Melki R, Didry D, Xia GX, Hong Y, Chua NH, Pantaloni D (1997). Actin depolymerizing factor ADF/cofilin enhances the rate of filament turnover: implication in actin-based motility. J Cell Biol. 136: 1307-1323.

Ceccarelli S, Grando S (1996). Drought as a challenge for the plant breeder. Plant growth regulation. 20: 149-155.

Cejudo FJ, Murphy G, Chinoy C, Baulcombe DC (1992). A gibberellin-regulated gene from wheat with sequence homology to cathepsin B of mammalian cells. Plant J. 2: 937-948.

Chazen O, Neumann PM (1994). Hydraulic signals from the roots and rapid cell-wall hardening in growing maiz (*Zea mays* L.) leaves are primary responses to polyethylene glycol-induced water deficits. Plant physiol. 104: 1385-1392.

Condon AG, Farquhar GD, Richards RA (1990). Genotypic variation in

carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. Aust. J. Plant Physiol. 17: 9-22.
Conley TR, Peng HP, Shih MC (1999). Mutations affecting induction of glycolytic and fermentative genes during germination and environmental stresses in Arabidopsis. Plant Physiol. 119: 599-608.
Cornic G, Massacci A (1996). In Advances in Photosynthesis, V.5, Photosynthesis and the Environment (Neil R. Baker, ed), pp. 347-366. Kluwer Academic Publishers, Dordrecht/Boston/London
Datta SK (2002). Recent development in transgenic for abioltic stress tolerance in rice. JIRCAS working report. PP. 43-53.
De Vetten NC, Lu G, Ferl RJ (1992). A maize protein associated with the G-box binding complex has homology to brain regulatory proteins. Plant Cell. 4: 1295-1307.
De Vienne D, Leonardi A, Damerval C, Zivy M (1999). Genetics of proteome variation for QTL characterization: application to drought-stress responses in maize. J. Exp. Bot. 50: 303-309.
Dolferus R, Ellis M, De Bruxelles GL, Frevaskis B, Hoeren F, Dennis ES, Peacock WJ (1997). Strategies of gene action in *Arabidopsis* during hypoxia. Ann. Bot. 97: 21-31.
Drew MC (1997). Oxygen deficiency and root metabolism: Injury and acclimation under hyboxia and anoxia. Annu. Rev. Plant Physiol. Plant Biol. 48: 223-250.
Dumas B, Sailland A, Cheviet JP, Freyssinet GP (1993). Identification of barley oxalate oxidase as a germin-like CR Acad Sci Paris. 316: 793–798.
Edelman J, Jefford TG (1968). The mechanism of fructosan metabolism in higher plants as exemplified in Helianthus tuberosus. New Phytol. 67:517-531.
Ernez M, Lannoye R (1991). Quantification of physiological disorders in stressed plants. in Physiology-Breeding of Winter cereals for stressed Mediterranean environments. INRA ed., Paris. PP. 369-390.
Espinosa-Ruiz A, Belles JM, Serrano R, Culianez-Macia FA (1999). *Arabidopsis thaliana* AtHAL3: a flavoprotein related to salt and osmotic tolerance and plant growth. Plant J. 20: 529-539.
Ezaki B, Gardner RC, Ezaki Y, Matsumoto H (2000). Expression of aluminum-induced genes in transgenic *Arabidopsis* plants can ameliorate aluminum stress and/or oxidative stress. Plant Physiol. 122: 657-665.
Ezaki T, Kawamura Y, Li N, Li ZY, Zhao L, Shu S (2001). Proposal of the genera Anaerococcus gen. nov., Peptoniphilus gen. nov. and Gallicola gen. nov. for members of the genus Peptostreptococcus. Int. J. Syst. Evol. Microbiol. 51:1521-1528.
Falk S, Maxwell DP, Laudenbach DE, Huner NPA (1996). In Advances in Photosynthesis, V.5, Photosynthesis and the Environment (Neil R. Baker, ed), pp. 367-385. Kluwer Academic Publishers, Dordrecht/Boston/London.
Farquhar GD, Ehleringer JR, Hubick KT (1989). Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 503–537.
Farquhar GD, Richards RA (1984). Isotopic composition of plant carbon correlates with water-use-efficiency of wheat genotypes. Aust. J. Plant Physiol. 11: 539–552.
Firn RD, Friend J (1972). Enzymatic production of the plant growth inhibito, Xanthoxin. Planta 103: 263-266.
Fuks B, Schnell DJ (1997). Mechanism of protein transport across the chloroplast envelop. Plant Physiol. 114: 405-410.
Gahrtz M, Stolz J, Sauer N (1994). A phloem-specific sucrose-H1 symporter from Plantago major L. supports the model of apoplastic phloem loading. Plant J. 6: 697–706.
Giuliano G, Hoffman N, Ko K, Scolnik PA, Cashmore AR (1988). A light-entrained circadian clock controls transcription of several plant genes. EMBO J. 7: 3635–3642.
Guerrero FD, Jones JT, Mullet JE (1990). Turgor-responsive gene transcription and RNA levels increase rapisly when pea shoots are wilted. Sequence and expression of three inducible genes. Plant Mol. Biol. 15:11-26.
Haldane JBS (1919). The combination of linkage values, and the calculation of distance between linkage factors. J. Genet. 8: 299-309.
Halliwell B, Gutteridge JMC (1984). Free Radicals in Biology and Medicine 2nd edn. Clarendon Press, Oxford.
Havaux M, Ernez M, Lannoye R (1988). Correlation between heat tolerance and drought tolerance in cerealsdemonstrated by rapid chlorophyll fluorescence tests. J. Plant Physiol. 133: 555-560.
Heitz T, Bergey DR, Ryan CA (1997). A gene encoding a chloroplast-targeted lipoxygenase in tomato leaves is transiently induced by wounding, systemin, and methyl jasmonate. Plant Physiol. 114: 1085-1093.
Hendry GAF (1993). Evolutionary origins and natural functions of fructans: A climatological, biogeographic and mechanistic appraisal. New Phytol. 123: 3-14.
Hirose T, Imaizumi N, Scofield GN, Furbank RT, Ohsugi R (1997). cDNA cloning and tissue specific expression of a gene for sucrose transporter from rice (Oryza sativa L.). Plant Cell Physiol. 38:1389-1396.
Hurkman, W.J., Lane, B.G. and Tanaka, C.K. 1994. Nucleotide sequence of a transcript encoding a germin-like protein that is present in salt-stressed barley (Hordeum vulgare L.) roots. Plant Physiol 104:803–804
Hurkman WJ, Tao HP, Tanaka CK (1991). Germin-like polypeptides increase in barley roots during salt stress. Plant Physiol. 97: 366–374.
Im YJ, Han O, Chung GC, Cho BH (2001). Antisense expression of an arabidopsis ω-3 fatty acid desaturase gene reduces salt/drought tolerance in transgenic tobacco plants. Molecules and cells. 13: 261-271.
Ingram J, Bartels D (1996). The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. 47: 377–403.
Jarillo JA, Leyva A, Salinas J, Martinez-Zapater JM (1993). Low temperature induces the accumulation of alcohol dehydrogenase mRNA in *Arabidopsis thaliana*, a chilling-tolerant plant. Plant Physiol. 101: 833-837.
Kampfenekel KM, Van Montagu M, Inzé D (1995). Effect of iron excess on Nicotiana plumbaginifolia plants. Implications to oxidative stress. Plant Physiol. 107, 725–735.
Kato-Noguchi H (1999). Flooding induced increases in alcohol gehydrogenase activity in timothy and ryegrass seedlings. Biol. Plant. 42: 445-449.
Kato-Noguchi H (2000). Osmotic stress increases alcohol dehydrogenase activity in maize seedlings. Biol. Plant. 43: 621-624.
Kennedy RA, Rumpho ME, Fox TC (1992). Anaerobic metabolism in plants. Plant Physiol. 100:1-6.
Koizumi M, Yamaguchi-Shinozaki K, Tsuii H, Shinozaki K (1993). Structure and expression of two genes that encode distinct drought-inducible cysteine proteinases in Arabidopsis thaliana. Gene. 129:175-182.
Koroleva OA, Tomos AD, Farrar JF, Gallagher J, Pollock CJ (2001). Carbon allocation and sugar status in individual cells of barley leaves affects expression of sucrose-fructan 6-fructosyltransferase gene. Ann. Appl. Biol. 138:27–32.
Koroleva OA, Tomos AD, Farrar JF, Roberts P, Pollock CJ (2000). Tissue distribution of primary metabolism between epidermal, mesophyll and parenchymatous bundle cells in barley leaves. Aust. J. Plant Physiol. 27: 747–755.
Kuhn C, Franceschi VR, Schulz A, Lemoine R, Frommer WB (1997). Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. Science 275: 1298–1300.
Lane BG (1994). Oxalate, germin, and the extracellular matrix of higher plants. FASEB J. 8: 294–301.
Lane BG, Dunwell JM, Ray JA, Schmitt MR, Cuming AC (1993). Germin, a protein marker of early plant development, is an oxalate oxidase. J Biol. Chem. 268:12239–12242.
Lappalainen P, Fedorov EV, Fedorov AA, Almo SC, Drubin DG (1997). Essential functions and actin-binding surfaces of yeast cofilin revealed by systematic mutagenesis. EMBO J. 16: 5520–5530.
Lappaleinen P, Kessels MM, Cope MJTV, Drubin D (1998). The ADF homology (ADF-H) domain: a highly exploited actin-binding module. Mol. Biol. Cell. 9:1951–1959.
Leopold AC (1990). Coping with desiccation. In: Alscher RG and Cumming JR. Stress responses in plants: Adaptation and Acclimation Mechanisms. Wily-Liss, New York. pp. 37-56.
Leung H (2001). Genetic resources conservation, evaluation, and gene discovery. IRRI, project summary and highlights, pp. 13-16.

Lewis DH (1993). Nomenclature and diagrammatic representation of oligomeric fructans. New Phytologist. 124: 583-593.
Lidgett AJ, Moran M, Wong KAL, Furze J, Rhodes MJC, Hamill JD (1995). Isolation and expression pattern of a cDNA encoding a cathepsin B-like protease from Nicotiana rustica. Plant Mol. Biol. 29: 379-384.
Lu C, Koroleva OA, Farrar JF, Gallagher J, Pollock CJ, Deri TA (2002). Rubisco Small Subunit, Chlorophyll a/b-Binding Protein and Sucrose: Fructan-6-Fructosyl Transferase Gene Expression and Sugar Status in Single Barley Leaf Cells in Situ. Cell Type Specificity and Induction by Light. Plant Physiol. 130:1335–1348.
Ludlow MM, Chun ACP, Clements RT, Kerslake RG (1983). Adaptation of speccies of *Centrosema* to water stress. Aust. J. Plant Physiol. 10:119-130.
Manly KF, Cudmore Jr RH (1997). Map Manager QT, Software for mapping quantitative trait loci. Abstracts of the 11th International Mouse Genome Conference, St. Petersburg, FL.
Matsukura C, Saitoh T, Hirose T, Perata ROP, Yamaguchi J (2000). Sugar Uptake and Transport in Rice Embryo. Expression of Companion Cell-Specific Sucrose Transporter (OsSUT1) Induced by Sugar and Light. Plant Physiol. 124: 85-93.
McGough A, Chiu W (1999). ADF/cofilin weakens lateral contacts in the actin filament. J. Mol. Biol. 291: 513–519.
Merah O, Monneveux P, Dele´ens E (2001). Relationships between flag leaf carbon isotope discrimination and several morpho-physiological traits in durum wheat genotypes under Mediterranean conditions. Environ. Exp. Bot. 45: 63–71.
Nachit MM (1983). Use of planting dates to select stress tolerance and yield stable genotypes for the rainfed Mediterranean environment. Rachis. 3:15-17.
Nachit MM, Elouafi I, Pagnotta MA, El saleh A, Iacono E, Labhilili M, Asbati A, Azrak M, Hazzam H, Benscher D, Khairalla M, Ribaut JM, Tanzarella OA, Porceddu E, Sorrells ME (2001). Molecular linkage map for an interspecific recombinant inbred population of durum wheat (Triticum turgidum L. Var. Durum). Theo Appl. Genet. 102:177-186.
Nachit MM, Ketata H (1986). Breeding strategy for improving durum wheat in Mediterranean rainfed areas. International Wheat Conference, Rabat, Morocco, May. pp. 2-5.
Nachit MM, Ketata H (1991). Selection of morpho-physiological traits for multiple abiotic stresses resistance in durum wheat (Triticum turgidum L. var. Durum). In: Physiology-Breeding of Winter Cereals for Stressed Mediterranean Environments, (E. Acevedo, A.P. Conesa, Ph. Monneveux, J.P. Srivastava, Eds.). pp 391-400. Les
Colloques 55, INRA, Paris.
Nachit MM, Monneveux P, Araus JL, Sorrells, ME (2000). Relationship of dryland productivity and drought tolerance with some molecular markers for possible MAS in durum wheat (*Triticum turgidum* L. var. durum). Durum whaet improvement in the Mediterranean region: New challenges. pp. 203-206.
Nagai J, Bloch K (1968). Enzymatic desaturation of stearyl acyl carrier protein. J. Biol. Chem. 243: 4626–4633.
O'Tool JC (1989). Breeding for drought resistance in cereals: emerging new technologies. In: Baker FWG, ed. Drought resistance in cerials. Wallingford, UK: CAB international. pp. 81-94.
Ouellet FO, Carpentier E, Cope MJTV, Monroy AF Sarhan F (2001). Regulation of a Wheat Actin-Depolymerizing Factor during Cold Acclimation. Plant Physiol. 125: 360–368.
Ozturk NZ, Talame V, Michalowski CB, Gozukirmizi N, Tuberosa R, Bohnert HJ (2002). Monitoring large-scale changed in transcript abundance in drought- and saly-stressed barley. Plant molecular biology (In press).
Pilon-Smits EAH, Ebskamp MJM, Paul MJ, Jeuken JW, Weisbeek PJ, Smeekens SCM (1995). Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol. 107: 125–130.
Pollock CJ (1986). Fructans and the metabolism of sucrose in vascular plants. New Phytol. 104:1-24.
Pollock CJ, Cairns AJ 1991). Fructan metabolism in grasses and cereals. Annu. Rev. Plant Physiol. 42:77-101.
Pontis HG, Del CE (1985). Fructans, In: Dey PM, Dixon RA eds. Biochemistry of storage carbohydrates in green plants. Academic Press, New York, NY. pp. 205-227.
Rekika D, Nachit MM, Araus JL, Monneveux P (1998). Effects of water deficit on photosynthetic capacity and osmotic adjustment in tetraploid wheats. Photosynthetica. 129-138.
Reynolds MP, Balota M, Delgado MIB, Amani I, Fischer RA (1994). Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Aust. J. Plant Physiol. 21, 717–730.
Ricard B, Couee I, Raymond P, Saglio PH, Saint-Ges V, Pradet A (1994). Plant metabolism under hypoxia and anoxia. Plant Physiol. Biochem. 32:1-10.
Richards RA, Condon AG (1993). Challenges ahead in using carbon isotope discrimination in plant breeding programs. In 'Stable isotopes and plant carbon/water relations'. (Eds JR Ehleringer, AE Hall, GD Farquhar) pp. 451–462. (Academic Press: New York).
Roxas VP, Smith RK, Allen ER, Allen RD (2000). Overexpression of glutathione-S-transferase/glutathione peroxidase enhances the growth of transgenic tobacco seedlings during stress. Nature Biotechnol. 15: 988-991.
Royo C, Villegas D, García del Mora LF, Elhani S, Aparicio N, Rharrabti Y, Araus JL (2002). Comparative performance of carbon isotope discrimination and canopy temperature depression as predictors of genotype differences in durum wheat yield in Spain. Aust. J. Agric. Res. 53:561–569.
Royo C, Garcia Del Moral LF, Aparicio N, Villegas D, Casadesus J, Araus JL (2000). Tools for improving the efficiency of durum wheat selection under Mediterranean conditions. Durum wheat improvement in the Mediterranean region: New challenges. pp. 63-70.
Reynolds, M.P., Singh, R.P., Ibrahim, A., Ageeb, O.A., Larqué-Saavedra, A., and Quick J.S., 1998. Evaluating physiological traits to complement empirical selection for wheat in warm environments. Euphytica 100:85-94.
Sauer N, Stolz J (1994). SUC1 and SUC2: two sucrose transporters from Arabidopsis thaliana. Expression and characterization in baker's yeast and identification of the histidine-tagged protein. Plant J. 6: 67–77.
Sindhu R, Walton DC (1987). The conversion of xanthoxin to abscisic acid by cell-free preparations from bean leaves. Plant Phsiol. 85: 916-921.
Sprenger N, Bortlik K, Brandt A, Boller T, Wiemken A (1995). Purification, cloning, and functional expression of sucrose:fructa- 6-fructosyltransferase, a key enzyme of fructan synthesis in barley. Proc. Natl. Acad. Sci. U.S.A. 92: 11652-11656.
Staiger CJ, Gibbon BC, Kovar DR, Zonia LE (1997). Profilin and actin-depolymerizing factor: modulators of actin organization in plants. Trends Plant Sci. 2: 275–281.
Tambussi EA, Casadesus J, Munné-Bosch S, Araus JL (2002). Photoprotection in water-stressed plants of durum wheat (*Triticum turgidum* var. *durum*): changes in chlorophyll fluorescence, spectral signature and photosynthetic pigments. Funct. Plant Biol. 29:35–44.
Tetlow IJ, Farrar, JF (1993). Apoplastic sugar concentration and pH in barley leaves infected with Brown rust. J. Exp. Bot, 4: 929-936.
Van Deynze AE, Nelson JC, Harrington SE, Yglesias ES, Braga D, McCouch SR, Sorrells ME (1995b). Comparative mapping in grasses: wheat relationships. Mol. Gen. Genet. 248: 744-754.
Vick BA, Zimmerman DC (1987). oxidative systems for modification of fatty acids: the lipoxygenase pathway. In: Stumpf PK (ed) The biochemistry of plants: A comprehensive treatise, Vol 9. Academic Press, New York. pp. 53-90.
Villegas D, Aparicio N, Nachit MM, Araus JL, Royo C (2000). Photosynthetic and developmental traits associated with genotypic differences in durum wheat yield across the Mediterranean basin. Aust. J. Agric. Res. 51:891–901.
Vincent JL, Dahiya P, Brewin NJ (2000). Localized expression of cathepsin B-like sequences from root nodule of Pea (Pisum sativum). American Phtopathology Society. 13: 778-780.
Walker-Simmons MK (1997). The wheat protein kinase gene, TaPK3, of the PKABA1 subfamily is differentially regulated in greening wheat seedlings. Plant Mol. Biol. 33: 935-941.
Ward W, Alvarado L, Rawlings ND, Engel JC, Franklin C, McKerrow JH (1997). A primitive enzyme for a primitive cell: The protease required

for excystation of Giardia. Cell 89: 437-444.
Wei Y, Zhang Z, Andersen CH, Schmelzer E, Gregersen PL, Collinge DB, Smedegaard-Petersen V, Thordal-Christensen H (1998). An epidermis/papilla-specific oxalate oxidase-like protein in the defence
response of barley attacked by the powdery mildew fungus. Plant Mol. Biol. 36: 101–112.
Williams ML, Farrar JF, Pollock CJ (1989). Cell specialisation within the parenchymatous bundle sheath of barley. Plant Cell Environ. 12: 909–918.
Wilson JR, Fisher MJ, Schulze ED, Dolby GR, Ludlow MM (1997). Comparison between pressure-volume and lew point-hygrometry techniques for determining water relation characteristics of grass and legume leaves Oecologia. 41: 77-88.
Yang KY, Im YJ, Chung GC, Cho BH (2002). Activity of the Arabidopsis blue copper-binding protein gene promoter in transgenic tobacco plants upon wounding. Plant Cell Rep. 20: 987–991.
Zhou F, Zhang Z, Gregersen PL, Mikkelsen JD, de Neergaard E, Collinge DB, Thordal-Christensen H (1998) Molecular Characterization of the Oxalate Oxidase Involved in the Response of Barley to the Powdery Mildew Fungus. Plant Physiol. 117: 33-41.