Genes, Genomes and Genomics ©2007 Global Science Books

Comparative Genetics of Stress-Related Genes and Chromosomal Regions Associated with Drought Tolerance in Wheat, Barley and Rice

Ayman A. Diab[1*] • Ramesh Kantety[2] • Carlos Mauricio La Rota[2] • Mark E. Sorrells[2]

[1] Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center, Giza, Egypt
[2] Department of Plant Breeding, 252 Emerson Hall, Cornell University, Ithaca, NY, USA
Corresponding author: * **adiab@msa.eun.eg**

ABSTRACT

An integrated barley consensus map was constructed and used with durum wheat and rice maps to develop comparative genetic maps for durum wheat, barley and rice. Comparative maps were constructed in three stages, each adding a new layer of information. In the first stage, comparative maps were constructed based on common markers present in durum wheat (A and B genomes) and barley genomes compared to rice maps. In the second stage, the marker sequences were matched according to sequence similarity across species. In the third stage, the sequences of drought candidate genes and differentially expressed sequence tags (dESTs) were compared to Bacterial Artificial Chromosome /Phage Artificial Chromosome sequences of the rice genome. The analysis of stress-related genes and dESTs in durum wheat, barley and rice revealed that the genetic response to drought stress is partially conserved among these species. Comparative maps identified conserved genomic regions that are associated with quantitative trait loci (QTLs) for drought tolerance in durum wheat, barley and rice. Some QTLs were unique to only one species, whereas other QTLs for related traits were co-located in all three species.

Keywords: Expressed sequence tags, *in silico* mapping, linkage map, Quantitative trait loci, recombinant inbred lines, Simple sequence repeats, synteny

Abbreviations: BAC, Bacterial Artificial Chromosome; **dEST**, differential expressed sequence tags; **EST**, expressed sequence tags; **QTLs**, quantitative trait locus; **RFLP**, Restriction Fragment Length Polymorphism; **RILs**, recombinant inbred lines; **SSR**, Simple sequence repeats

CONTENTS

INTRODUCTION

Quantitative trait loci (QTLs) mapping, gene cloning, expressed sequence tags (ESTs) and genome sequencing projects have led to a vast body of genetic information in public databases and provided the scientific community with powerful tools for comparative genomics (Gai *et al*. 2000; Mekhedov *et al*. 2000). The development of comparative maps allows scientists to compare genetic information from diploid species with small genomes, such as rice, to species with more complex genomic structure, such as wheat, and increases the efficiency of molecular-marker and gene isolation technologies applied to crop improvement. The integration of genetic information from related species could lead

Received: 6 October, 2006. Accepted: 27 February, 2007.

Invited Review

to the identification of highly conserved sequences and/or regulatory mechanisms by which it is possible to predict the function and location of genes in different organisms that have traditionally been studied separately.

Molecular markers have been used to develop comparative chromosome maps for several members of the Gramineae (Van Deynze *et al*. 1995a, 1995b, 1995c; Devos and Gale 1997) and these have been used to study genes of agronomic importance across species (for review see Snape and Laurie 1996). Most comparative mapping among the grasses has relied on Restriction Fragment Length Polymorphism (RFLP) probes (cDNAs or genomic clones) to establish gross gene orders and distance in specific chromosome segments. Only to a limited extent have researchers employed cloned genes, ESTs, or QTLs in comparative genomics. Identification of chromosomal locations of ESTs and candidate genes facilitates the construction of more comprehensive comparative maps for grass genomes. This is particularly important for linking barley and wheat with the rice genome, because rice was the first grass genome to be sequenced.

Drought stress is highly heterogeneous in time (over the seasons and years) and space (between and within sites), and is unpredictable. The diversity of drought tolerance strategies employed by plants, that may be targeted and used as selection criteria, makes it difficult to identify or simulate a representative drought stress condition. However, substantial progress has been made in recent years, on different plant species, in terms of physiology, genetics and molecular biology of drought tolerance.

In several cereal species, genetic maps have allowed the identification of chromosomal regions controlling some traits related to drought stress response. Many morphological and physiological characters are known to be involved in drought tolerance. Several segregating populations from rice, barley and wheat have been studied for different quantitative characters such as root characters, photosynthesis parameters, chlorophyll content, water-use efficiency, carbon isotope discrimination, water status and osmotic adjustment parameters. Champoux *et al*. (1995), Lilley *et al*. (1996), Ray *et al*. (1996), Redona and Mackill (1996), Price *et al*. (1997), and Li *et al*. (1999) have mapped QTLs for leaf rolling, root-related parameters, osmotic adjustment and relative leaf water content traits in rice. Quantitative trait loci for relative water content, osmotic adjustment, osmotic potential, water soluble carbohydrate concentration and carbon isotope discrimination have been identified in barley recombinant inbred lines (RILs) population derived from a cross between Tadmor and Er/APM (Teulat *et al*. 1998, 2001). Using the same population, Diab *et al*. (2004) reported the colocation of differentially expressed sequence tags and candidate genes with QTLs for water soluble carbohydrate, accumulation of water soluble carbohydrate at full turgor, osmotic potential and relative water content. In a durum wheat population, derived from the cross between Jennah Khetifa and Cham1, Diab *et al*. (2003) identified locations of drought candidate genes and differentially expressed transcripts and associated some of them with the QTLs for photosynthesis parameters, chlorophyll content, water use efficiency, carbon isotope discrimination, water status and osmotic adjustment parameters. Morgan and Tan (1996) also mapped a major gene for osmoregulation in bread wheat.

The analysis of drought stress response can be carried out by sequence comparison of stress-related genes and by looking for drought tolerance traits among regions of colinearity in related genomes. Matching of mapped wheat and barley probes (cDNAs, genomic clones, ESTs and candidate genes) with the rice genome sequence will identify putative orthologous loci between these genomes and will facilitate the transfer of information on a QTLs or a known gene function between grass species.

Despite the progress in comparative mapping, the application of this technology, especially for wheat and barley will not be realized unless scientifically sound strategies for studying drought tolerance are devised that allow researchers to utilize genetic tools and information developed for model species such as rice. This requires more detailed comparative genetic analysis from the DNA sequence of genes all the way to comparative analysis of QTLs.

The objective of this work was to identify conserved chromosomal regions and stress-related gene sequences that are involved in drought tolerance in wheat, barley and rice genomes through comparative analysis using sequence and map-based tools. Because of extensive colinearity among Triticeae genomes, wheat and barley in particular, the results presented in this work can be extended to other Triticeae genomes.

MATERIALS AND METHODS

Genetic linkage maps and drought QTLs

Linkage maps (with drought-related QTLs) of durum wheat, barley and rice were employed to identify conserved genomic regions associated with drought tolerance among these species.

Durum wheat map

A mapping population of 110 F_9 RILs derived from two durum wheat (*Triticum turgidum* L. var durum) parents Jennah Khetifa and Cham1 (JK/Cham1) that exhibit contrasting traits for drought tolerance was employed. Candidate drought genes and differentially expressed barley sequences were used to produce an integrated genetic linkage map containing 468 markers. Map construction, identification of chromosomal locations and genetic contributions of genes controlling traits related to drought tolerance in this population have been previously described by Diab *et al*. (2003).

Barley map

A segregating population of 167 barley (*Hordeum vulgare* L.) RILs derived from a cross between Tadmor and Er/APM (Tad/Er/APM) was used. An integrated genetic linkage map containing 166 loci, including drought genes and differentially expressed sequence tags (dESTS), was constructed and QTLs affecting variation in drought traits were identified. Linkage map construction and identification of QTLs have been previously described by Diab *et al*. (2004), Teulat *et al*. (1998) and Teulat *et al*. (2001).

Rice map

The Cornell rice RFLP 2001 map (http://www.gramene.org) was downloaded and employed. This map is an updated version of the Cornell RFLP 1994 map reported by Causse *et al*. (1994) and revised by Wilson *et al*. (1999). The mapping population was derived from a backcross between cultivated rice, *Oryza sativa*, and its wild African relative, *Oryza longistaminata*. Quantitative trait loci related to drought tolerance in rice were gathered from different rice studies and integrated into this map for the purpose of QTLs comparison (**Table 1**).

Construction of barley consensus map

Two segregation data sets, Tadmor X Er/APM (Diab *et al*. 2004) and barley consensus map (Qi *et al*. 1996) were downloaded from the publicly available GrainGenes database (http://www.graingenes.org). To integrate the Tad/Er/APM map into a barley consensus map, each linkage group was divided into bins based on the common markers between the two maps. Using the common markers as a border for each respective bin, the genetic distance between the markers within each bin was converted to a percent fraction of the total bin length. Then markers from the Tad/Er/APM map were integrated based on the percent fractions. The QTLs that were identified in the Tad/Er/APM population were then transferred to the integrated consensus map.

Construction of comparative maps

The comparative maps were constructed in three stages with each

Table 1 Quantitative trait loci for drought related traits that are gathered from different rice studies and placed on rice Cornell RFLP 2001 linkage map for QTLs comparison purpose.

Trait	QTLs	Marker	chromosome	Reference
Relative water content	RWC	RG182	5	Lilley *et al.* 1996
		C624	5	Price *et al.* 2002
		C734	4	Price *et al.* 2002
Root thickness	Rthic	C624	5	Price *et al.* 1997
		RZ892	10	Ali *et al.* 2000
		BCD386	10	Ali *et al.* 2000
		RG939	4	Zhang *et al.* 2001
		RG9	1	Zhang *et al.* 2001
		WG110	1	Ali *et al.* 2000
		CDO464	8	McCouch and Doerge 1995
Leaf rolling	LR	C624	5	Price *et al.* 2002
		RZ892	10	Champoux *et al.* 1995
		RZ400	10	Champoux *et al.* 1995
		RZ69	4	Champoux *et al.* 1995
		RG462	1	Champoux *et al.* 1995
		RZ14	1	Price *et al.* 2002
		RG96	3	Champoux *et al.* 1995
		GR910	3	Champoux *et al.* 1995
		CDO475	6	Champoux *et al.* 1995
		RZ398	6	Champoux *et al.* 1995
		CDO595	8	McCouch and Doerge 1995
		CDO464	8	McCouch and Doerge 1995
Root penetration index	Rpent	RZ892	10	Ali *et al.* 2000
		BCD386	10	Ali *et al.* 2000
		RG939	4	Zhang *et al.* 2001
		RG9	1	Zhang *et al.* 2001
Root length	RL	RG400	1	Price *et al.* 1997
Osmotic adjustment	OA	RG140	1	Zhang *et al.* 2001
Osmotic potential	OP	RG109	1	Lilley *et al.* 1996
		RG96	3	Lilley *et al.* 1996
Cell membrane stability	CMS	CDO34	1	Tripathy *et al.* 2000
		RZ403	3	Tripathy *et al.* 2000

stage adding a new layer of information. In the first stage, comparative maps were constructed based on common markers (anchor loci) present in durum wheat (A and B) and barley genomes compared to rice maps. In the second stage, the marker sequences were matched according to sequence similarity and this information was used to identify additional links between the three species. Finally, we compared the sequences of candidate genes and differentially expressed transcript sequences to BAC/PAC sequences of the rice genome and were able to identify rice markers associated with the same BAC/PACs clone.

Construction of framework comparative map

A relational database (MySQL) was created consisting of the integrated barley consensus and JK/Cham1 maps in addition to the Gramineae maps downloaded from GrainGenes (http://www.graingenes.org) and Gramene (http://www.gramene.org) databases. The integrated barley consensus and JK/Cham1 maps were compared against themselves and to other rice maps based on common marker/probe names. This information was used to construct framework comparative maps between durum wheat, barley and rice.

Sequence similarity of probes

A total of 4844 probe sequences mapped in grass genomes were downloaded from GenBank and GrainGenes databases. In addition, 112 sequences of candidate genes and dESTs were downloaded from GenBank. The sequences were subjected to masking of known repeat sequences from the Poaceae family (MITES, etc.) as well as low-complexity sequences, including the SSRs and interspersed repeats, using the RepeatMasker Program (Smit 1999). The masked sequences were then compared against each other using NCBI BLASTN tool (Altschul *et al.* 1997) with an e-value threshold of 1 e^{-10}. The results of these sequence comparisons were parsed, uploaded to the relational database and further filtered to allow for hits of 80% similarity or greater, over 50 base pairs and having at least half of each sequence present in the alignment. The integrated barley consensus and JK/Cham1 maps were then compared against each other and the rice maps. The information obtained from this stage was integrated with the framework comparative map.

In silico mapping of candidate genes and dEST

One hundred and twelve sequences belonging to candidate genes and dESTs were compared to 3,406 rice BAC/PAC sequences using BLAST (with an e-value threshold of $1e^{-10}$). The rice BAC/PAC name that matches each query dEST or candidate gene was used to identify anchored rice markers from the Cornell rice RFLP map (http://www.tigr.org/tdb/e2k1/osa1/BACmapping/description.shtml).

The results obtained from this stage were integrated with those obtained from the previous two stages to construct a final comparative map between durum wheat, barley and rice. **Figs. 1** through **6** show the comparative maps of the conserved regions containing significant QTLs associated with drought tolerance in the three species.

RESULTS AND DISCUSSION

QTLs for drought tolerance in durum wheat, barley and rice

The genetic and molecular dissection of drought tolerance has led to the identification of either genomic regions involved in drought tolerance (QTLs), or DNA sequences known to play a role in molecular stress responses (stress related genes, differentially expressed sequences).

In the durum wheat population Cham 1 x Jennah Ketifa, the most significant QTLs were obtained for canopy temperature depression, photosynthesis-related parameters and

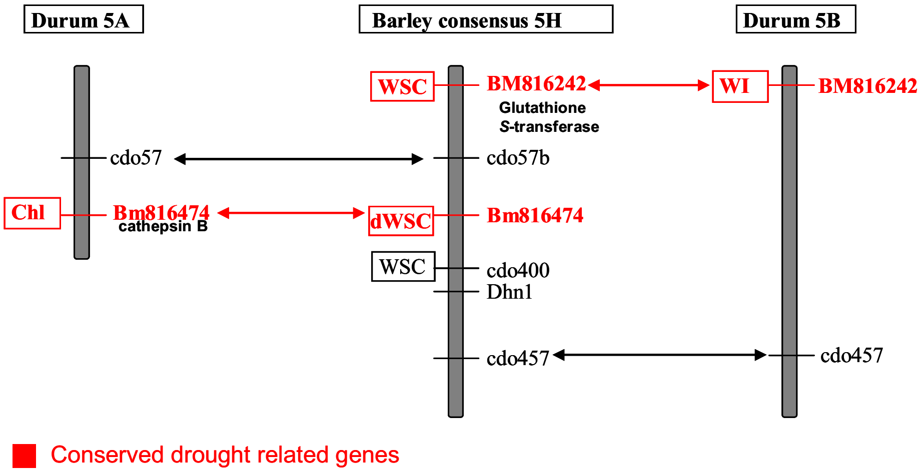

Fig. 1 Conserved genomic regions for drought related traits between durum wheat (chromosomes 5A and 5B) and integrated barley consensus 5H. For clarity only partial chromosomes were drawn. Vertical bars beside each chromo-some indicate the position of QTLs for chlorophyll content (Chl), water soluble carbohydrates (WSC), accumulation of WSC at full turgor (dWSC) and water index (WI).

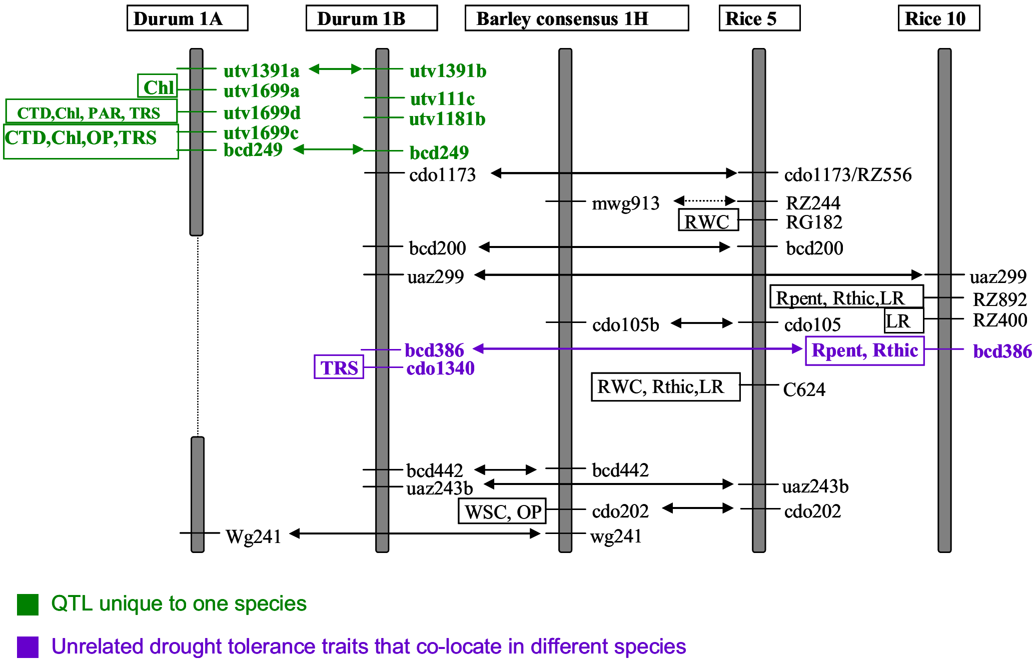

Fig. 2 Conserved genomic regions for drought related traits between durum wheat (chromosomes 1A and 1B), integrated barley consensus 1H and rice 5 and 10. For clarity only partial chromosomes were drawn. Dotted arrows indicate that the markers were matched using sequence similarity. Vertical bars beside each chromosome indicate the position of QTLs for canopy temperature depression (CTD), photosynthetic active radiation (PAR), transpiration (TRS), chlorophyll content (Chl), relative water content (RWC), water soluble carbohydrates (WSC), osmotic potential (OP), root peneration indix (Rpent), leaf rolling (LR) and root thickness (Rthic).

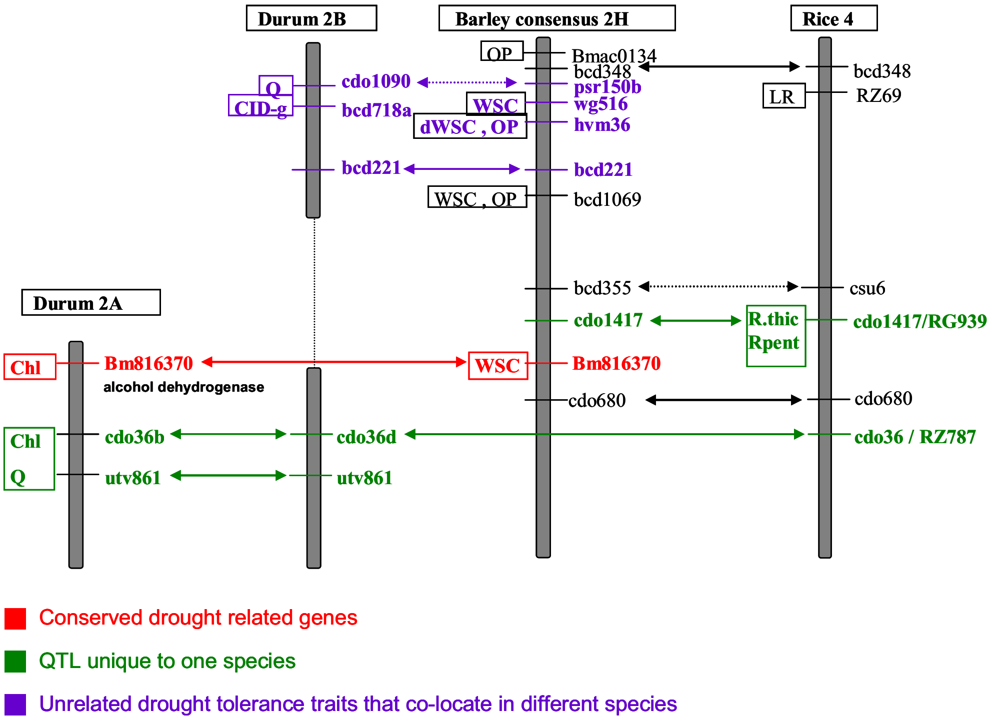

Fig. 3 Conserved genomic regions for drought related traits between durum wheat (chromosomes 2A and 2B), barley consensus 2H and rice 4. For clarity only partial chromosomes were drawn. Dotted arrows indicate that the markers were matched using sequence similarity. Vertical bars beside each chromosome indicate the position of QTLs for chlorophyll content (Chl), quantum yield (Q), grain carbon isotope discrimination (CID-g), water soluble carbohydrates (WSC), accumulation of WSC at full turgor (dWSC), osmotic potential (OP), leaf rolling (LR), root penetration index (Rpent) and root thickness (Rthic).

water status traits (Diab 2003). One hundred and seventy eight different chromosome regions including 6 candidate genes and 19 differentially expressed sequences were associated with QTLs for various drought tolerance traits.

In barley, a total of 68 QTLs were reported for relative water content, osmotic adjustment, osmotic potential, water soluble carbohydrate concentration and carbon isotope discrimination (Teulat *et al.* 1998, 2001; Diab *et al.* 2004). For the same population, forty-eight markers, including candidate genes and differentially expressed sequences were associated with QTLs for drought tolerance traits (Diab *et al.* 2004).

In rice, QTLs for relative water content, described by Lilley *et al.* (1996) and Price *et al.* (2002), root thickness (McCouch and Doerge 1995; Price *et al.* 1997; Ali *et al.* 2000; Zhang *et al.* 2001), leaf rolling (Champoux *et al.* 1995; McCouch and Doerge 1995; Price *et al.* 2002), root penetration index (Ali *et al.* 2000; Zhang *et al.* 2001), root length (Price *et al.* 1997), osmotic adjustment (Zhang *et al.* 2001), osmotic potential (Lilley *et al.* 1996) and cell membrane stability (Tripathy *et al.* 2000) were assigned to 23 corresponding markers in the Cornell rice RFLP 2001 map (**Table 1**). Seven markers were found to be associated with more than one trait. Some of these traits have been identified in different rice studies (**Table 1**). For example, QTLs for leaf rolling and osmotic potential was assigned to the same chromosomal region (RG96) on chromosome 3 (from Champoux *et al.* 1995 and Lilley *et al.* 1996) and QTLs for root thickness, root penetration index and leaf rolling associated with the locus RZ892 on chromosome 10 (from Champoux *et al.* 1995 and Ali *et al.* 2000). Similarly, QTLs for relative water content, root thickness and leaf rolling were found in the same chromosomal region (C624) on chromosome 5 (Price *et al.* 2002). The overlap of these QTLs suggests a possible common genetic control.

Integrated barley consensus map

The 166 loci of the Tad/Er/APM map were merged with the 880 loci of the previously published consensus map to pro-

Table 2 Comparison of number of anchor links between genomes for three stages of comparative map construction for durum wheat, barley and rice.

Comparative Map	Number of common markers		
	1st stage (Frame work map)	2nd stage (Sequence similarity)	3rd stage (*in silico* mapping)
Tad/Er to JK/Cham1	13	13	-
IBC to JK/Cham1	26	32	-
Tad/Er to RC-2001	6	7	13
IBC to RC-2001	27	41	59
JK/Cham1 to RC-2001	28	33	61

Tad/Er: Barley linkage map (Diab *et al.* 2004), JK/Cham1: durum wheat linkage map (Diab 2003), IBC: integrated barley consensus map constructed in this work, RC-2001: rice Cornell RFLP map 2001 (updated version of the Cornell RFLP 1994 map reported by Causse *et al.* 1994).

duce an integrated barley consensus map with 1011 markers (figures not shown). Thirty-five markers were found to be common between the two maps. Two markers were anchored for 1H, 7 for 2H, 5 for 3H, 4 for 4H, 8 for 5H, 3 for 6H and 6 for 7H. Comparison of the integrated map and the Tad/Er/APM map revealed that the overall linear order of markers was in good agreement. The total number of shared markers between barley and durum wheat increased from 13 to 32 when the integrated barley consensus map was compared to the Tad/Er/APM map. The shared markers between barley and rice increased from 6 to 59 (**Table 2**). These results meet with the main objective of constructing an integrated barley consensus map to increase the chances of finding anchor loci between durum wheat, barley and rice. This integrated barley consensus map, containing dESTs and candidate genes, should be useful for more precise mapping of cereals in general.

Stepwise construction of the comparative maps

The results obtained in this study indicate that the stepwise construction of the comparative map effectively increased the number of common probes between durum wheat, barley and rice. For example, the number of common markers between JK/Cham1 and rice maps increased from 28 in the first stage to 61 in the third stage (**Table 2**).

The first stage (framework map) resulted in 81 total common markers between the three species. This number increased to 106 when information about the sequence similarity of probes was integrated into the framework map. When the results of the third stage (*in silico* mapping of candidate genes and dEST) were integrated into those obtained from the previous two stages the total common markers between durum wheat, barley and rice increased to 120 (**Table 2**). Comparative map loci that deviated from the previously published comparative maps between wheat and rice (van Deynze *et al.* 1995a) and barley and rice (Sherman *et al.* 1995; Saghai Maroof *et al.* 1996) were noted and analyzed separately. The third stage enabled us to locate 43 out of 72 dESTs and genes that had not been mapped in durum wheat or barley due to the lack of polymorphism. They were located on the rice RGP map by sequence matching these genes and dESTs to rice BAC/PAC sequences.

Description of the comparative map

Comparative studies of RFLP maps between cereal species have shown conservation of genome structure (Chao *et al.* 1989; Devos *et al.* 1993; Devos and Gale 1993; van Deynze *et al.* 1995a). A more extensive analysis of genome organization (Moore *et al.* 1995) has revealed that the genome of rice can be subdivided into 19 linkage segments, which can be aligned with the genomes of wheat and barley.

Based on previous comparative linkage mapping studies, the rice linkage groups 5 and 10 are known to be syntenic with at least parts of the linkage group 1(1H), while the rice chromosome 1 is syntenic with chromosome 3(3H). Similarly, rice chromosomes (4 and 7), (3 and 10) and (6 and 8) are syntenic with chromosomes 2(2H), 4(4H), and 7(7H) respectively. **Figs. 1** through **6** demonstrate that the results of this study agree with expectations based on previous comparative linkage mapping studies. Despite substantial differences in DNA content and basic chromosome numbers, colinearity of genes was shown to be widespread across the genomes of durum wheat, barley and rice. However, as map data accumulate, it becomes increasingly difficult to find segments in which gene content and order are strictly parallel in the three genomes, due in part to experimental error, but also to high rates of insertion and deletion of small regions of chromosomes. For instance, we found colinear markers between 5A and 5B of durum wheat to 5H of barley but there was little or no conservation of gene order with rice (**Fig. 1**). This inability to relate the genomic structure of wheat and barley to specific rice chromosomes (or chromosomal regions) may also be due to a lack of comparative markers between them.

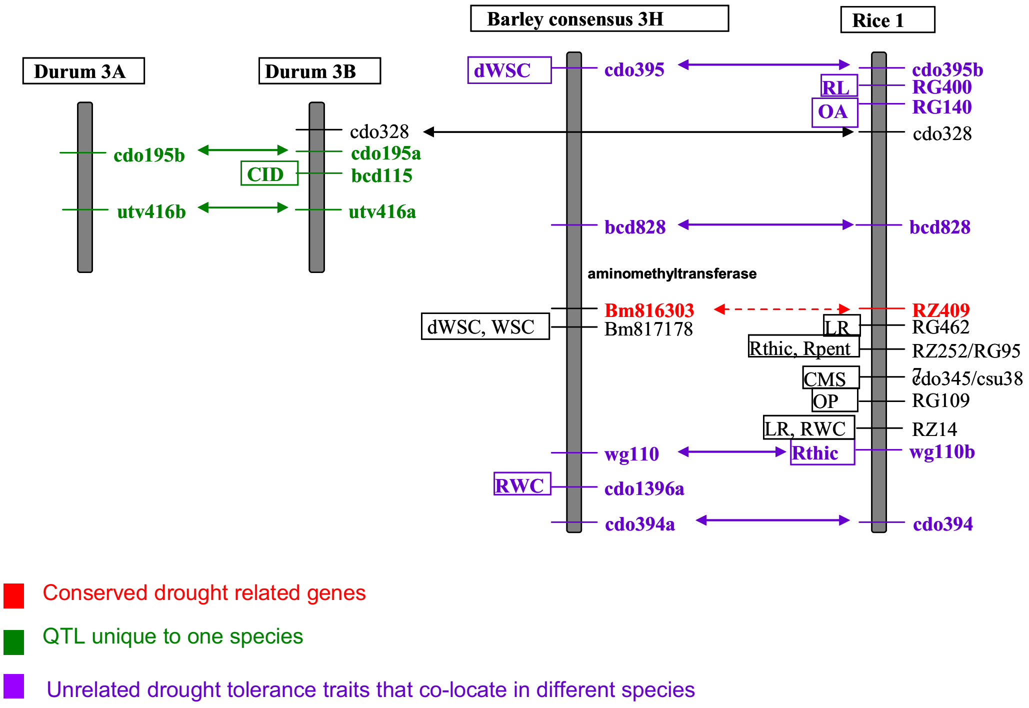

Fig. 4 Conserved genomic regions for drought related traits between durum wheat (chromosomes 3A and 3B), barley consensus 3H and rice 1. For clarity only partial chromosomes were drawn. Dashed arrows indicate transitive matches between genes/dEST and a marker associated to rice BAC/PAC. Vertical bars beside each chromosome indicate the position of QTLs for carbon isotope discrimination (CID), relative water content (RWC), water soluble carbohydrates (WSC), accumulation of WSC at full turgor (dWSC), osmotic potential (OP), osmotic adjustment (OA), leaf rolling (LR), root thickness (Rthic), root length (RL), root penetration index (Rpent) and cell membrane stability (CMS).

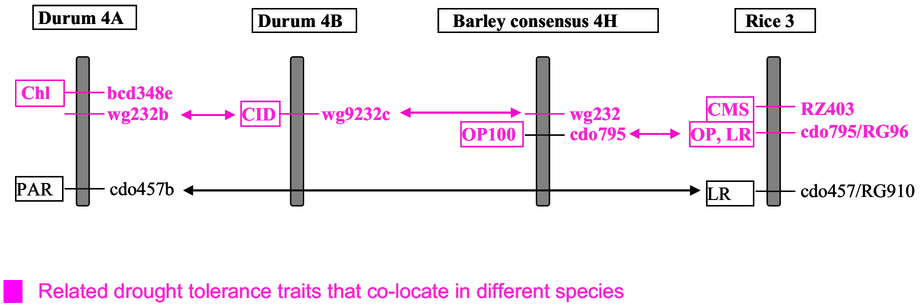

Fig. 5 Conserved genomic regions for drought related traits between durum wheat (chromosomes 4A and 4B), barley consensus 4H and rice 3. For clarity only partial chromosomes were drawn. Vertical bars beside each chromosome indicate the position of QTLs for chlorophyll content (Chl), photosynthetic active radiation (PAR), carbon isotope discrimination (CID), osmotic potential at full turgor (OP100), osmotic potential (OP), cell membrane stability (CMS) and leaf rolling (LR).

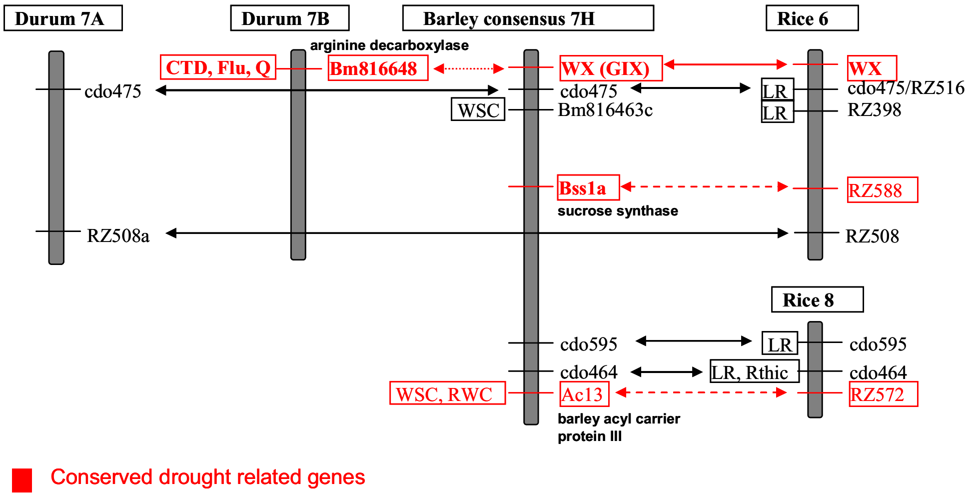

Fig. 6 Conserved genomic regions for drought related traits between durum wheat (chromosomes 7A and 7B), barley consensus 7H and rice 6 and 8. For clarity only partial chromosomes were drawn. Dashed arrows indicate transitive matches between genes/dEST and a marker associated to rice BAC/PAC. Vertical bars beside each chromosome indicate the position of QTLs for osmotic adjustment (OA), grain carbon isotope discrimination (CID-g), canopy temperature depression (CTD), Fluorescence index (Fluo), quantum yield (Q), water soluble carbohydrates (WSC), relative water content (RWC), leaf rolling (LR) and root thickness (Rthic).

Conserved genes and conserved function

Many fundamental aspects of cellular metabolism and pathways are conserved among eukaryotes, reflecting their early origin and importance to cell viability. The analysis of stress-related genes and dEST in durum wheat, barley and rice reveals that the molecular response to drought stress is at least partially conserved among these species.

The gene BM816474, coding for cathepsin B (Ozturk *et al.* 2002) that was associated with QTLs for chlorophyll content on chromosome 5A in durum (Diab 2003), was also mapped in barley 5H (**Fig. 1**) and cosegregated with QTLs for accumulation of water soluble carbohydrates (Diab *et al.* 2004). These two traits are components of drought tolerance and are correlated under drought stress (Nachit and Ketata 1991; Rekika *et al.* 1998; Teulat *et al.* 2001). Diab *et al.* (2003) suggested that a possible role for cathepsin B in maintaining chlorophyll content might involve the degradation of proteins that cause cell damage in response to drought stress. Another gene (BM816242), coding for slutathione *S*-transferase or GST (Ozturk *et al.* 2002), co-segregated with a QTLs for water index on chromosome 5B (Diab 2003) and with water soluble carbohydrates in barley 5H (Diab *et al.* 2004). Water index and water soluble carbohydrates are also correlated traits and have been used for screening for drought tolerance in barley and durum wheat (Reynolds *et al.* 1994; Teulat *et al.* 2001). It is also reported that GSTs attach to lipid hydroperoxides, which are harmful compounds generated in plants exposed to various stresses (Plaisance and Gronwald 1999). Two GST proteins have been purified from sorghum that have the ability to detoxify lipid hydroperoxides (Plaisance and Gronwald 1999). The presence of this gene in both durum wheat and barley suggests that it might have a common role in protecting cells from toxic lipid hydroperoxides that are formed under drought stress conditions.

The gene BM816370, coding for alcohol dehydrogenase (Ozturk *et al.* 2002), was mapped in durum wheat 2A and barley 2H and co-segregated with chlorophyll content in durum wheat and with water soluble carbohydrates in barley as was observed for cathepsin B (Diab 2003, 2004; **Fig. 3**). Drought and temperature stresses induce 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 presence of this gene in durum wheat and barley and its association with those two traits indicates that this gene likely plays a common role in drought tolerance in both species. These results are supported by the work of Tarchini *et al.* (2000), who reported that the alcohol dehydrogenase sequence is conserved in rice and maize.

The gene BM816303, coding for aminomethyltransferase (Ozturk *et al.* 2002), on barley chromosome 3H was cross-matched to the sequence for RZ409, which is associated with a rice BAC on chromosome 1 (**Fig. 4**). BM816303 was closely mapped (5.7 cM) to the locus BM817178 (Diab *et al.* 2004), which codes for isocitrate dehydrogenase (Ozturk *et al.* 2002) and associated with QTLs for water soluble carbohydrates (Diab *et al.* 2004). The role of these differentially expressed transcripts in drought tolerance is not known yet, but the existence of these coding sequences in both barley and rice suggests a possible conserved mechanism for drought response in both species.

An interesting example of conserved gene function is the differentially expressed transcript (BM816648), coding for arginine decarboxylase (Ozturk *et al.* 2002) found to be orthologous in durum wheat, barley and rice (**Fig. 6**). This locus is associated with QTLs for canopy temperature depression, fluorescence index and quantum yield in durum wheat (Diab 2003). The correlation between these traits has been reported in durum wheat (Villegas *et al.* 2000; Merah *et al.* 2001; Royo *et al.* 2002; Tambussi *et al.* 2002), bread wheat (Richards and Condon 1993) and in barley (Acevedo 1993). Arginine decarboxylase is a key enzyme 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 co-location of this expressed sequence in the three species strongly suggests a common role in drought tolerance by reducing chlorophyll loss, and thus enhancing photosynthesis under drought stress conditions.

Two genes on barley chromosome 7H were cross-mapped to rice chromosomes 6 and 8 (**Fig. 6**). The sequence of the gene Bss1, coding for sucrose synthase (Sanchez de la Hoz *et al.* 1992), was cross-matched to the sequence for RZ588 on chromosome 6. 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 been widely observed as a mechanism for plants to cope with water deficit (Whittaker *et al.* 2001). The co-location of this gene in both barley and rice suggests a possible conserved role in drought tolerance though accumulation of water soluble carbohydrates and thus maintenance of the osmotic potential in both species under drought stress. The sequence of the gene Ac13 on 7H, coding for barley acyl carrier protein III (Hansen and Von Wettstein-Knowles 1991) was cross-matched to the sequence of RZ572, which is associated with a rice BAC on chromosome 8 (**Fig. 6**). The role of this gene 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 in both barley and rice.

One complication in comparative mapping is the possibility of convergent evolution, in which genes with equivalent functions have evolved independently in different lineages. This phenomenon has been clearly demonstrated in secondary metabolite synthesis (Pichersky and Gang 2000). In these cases, genes identified on the basis of similar effects on phenotype may be unrelated in sequence. Another complication for comparative mapping is that paralogous members of gene families may retain the same function (Alfenito *et al.* 1998; Pichersky and Gang 2000). In these cases the genes will be related in sequence but may not be colinear. This can complicate comparative map-based approaches to gene isolation from wheat and barley. If a trait can be mapped in a small genome species such as rice then cloning from that species is preferred. Genes in other species can then be isolated by sequence homology. If the trait is not mapped in the small genome species then this approach becomes much riskier because the relevant gene may be absent. In such cases, map-based cloning in diploid relatives of wheat or in barley may be preferred because the target gene is likely to be present. Even in these cases, comparative mapping can provide useful markers even if candidate genes are not identified.

Colinear regions associated with drought-related traits

Comparative genomics has shown that not only the candidate genes and dESTs are conserved, but the genomic regions conferring drought tolerance in durum wheat, barley and rice may also have a common ancestral origin. We identified genomic regions that are associated with QTLs for drought tolerance in durum wheat, barley and rice. Comparative QTLs analyses were classified three ways. Some QTLs were unique to only one species, whereas in other cases QTLs for different traits were co-located in more than one species. In the third category, QTLs for related traits were co-located in all three species.

QTLs unique to one species

Quantitative trait loci for five related traits (chlorophyll content, photosynthetic active radiation, canopy temperature depression, transpiration, and osmotic adjustment) were located between the interval utv1391a – bcd249 on durum wheat chromosome 1A. These two markers identify a homoeologous region in durum wheat chromosome 1B. However, no QTLs were identified in this region for barley or rice (**Fig. 2**). The marker interval cdo36b – utv861 on durum chromosome 2A was colinear with rice, but the only QTLs were for chlorophyll and quantum yield on durum chromosome 2A (**Fig. 3**). Similarly, the cdo1417-RG939 interval on rice chromosome 4 is associated with QTLs for root thickness and root penetration but in the corresponding region on barley 2H no QTLs related to drought tolerance were detected. Another interval delimited by the homoeologous markers, cdo195 and utv416, on durum chromosomes 3A and 3B was associated with a QTLs for carbon isotope discrimination (**Fig. 4**).

These results are not surprising due the fact that different drought tolerance related traits were measured in different studies using different species populations. The environments used for these studies were different in terms of their geographical locations and experimental conditions. The presence of QTLs in only one homoeologous group (e.g. Durum 1A or 1B) may be due to one or more of these following reasons: a) no allelic variation for the genes responsible for QTLs in one of the genomes, b) gene expression in polyploid genomes may be controlled such that only one copy of a gene or one genomic region is expressed, c) a gap in linkage groups due to low coverage of genome by markers and d) use of marker systems, such as AFLP, that do not allow the identification of homoeologous chromosome segments.

Drought tolerance traits that co-locate in different species

In some cases, QTLs for traits that do not seem to be mechanistically related, co-located in different species based on the comparative map. For example, QTLs for quantum yield and grain carbon isotope discrimination were located between the markers cdo1090 and bcd221 on durum chromosome 2B, and these markers were cross-matched to barley 2H with QTLs for water soluble carbohydrate and osmotic potential. The corresponding region on rice chromosome 4 was associated with a QTLs for leaf rolling (**Fig. 3**). Water soluble carbohydrate and osmotic potential may involve a mechanism related to rice leaf rolling, but these traits seem to involve a different aspect of drought tolerance compared to quantum yield and grain carbon isotope discrimination in wheat. The locus cdo395, which is associated with accumulation of water soluble carbohydrates on barley chromosome 3H, was cross-matched with rice chromosome 1 where QTLs for root length and osmotic adjustment have been mapped (**Fig. 4**). The same case occurred on barley chromosome 7H near BM816463c (a gene coding for blue copper-binding protein) and the orthologous region on rice chromosome 6 in the interval between cdo475 and Bss1a/RZ588 (**Fig. 6**). Root length is likely under different genetic control; however, accumulation of water soluble carbohydrates and osmotic adjustment may be affected by the same mechanism. Just proximal to cdo475 in durum, QTLs for canopy temperature depression, fluorescence index, and quantum yield cosegregated with BM816648 (a gene coding for arginine decarboxylase), but this seems to be a different locus from the nearby region in barley and rice. Thus, while these QTLs were located in homoeologous regions in the different species, they do not always seem to be mechanistically related. The underlying candidate genes may suggest a possible mechanism.

In durum chromosome 1B, the locus bcd386 is closely linked to the QTLs for transpiration efficiency and its orthologous region on rice chromosome 10 is associated with QTLs for root penetration index and root thickness. Another example of unrelated traits that are co-located in different species is represented by the interval wg110 – cdo394 on barley chromosome 3H with a QTL for relative water content. These two markers were cross-matched on rice chromosome 1 with a QTL for root thickness (**Fig. 4**). Root penetration ability and root thickness are two important parameters contributing to drought tolerance in rice

(Zhang *et al.* 2001). Roots are the main organs for plant water uptake, and well-developed deep root systems facilitate water uptake from lower soil layers and thus help plants to maintain better water status under drought stress. Confirmation of this relationship would require QTL mapping of root characters in the wheat and barley populations and evaluation of transpiration efficiency and relative water content in the rice population.

Related traits that co-locate in different species

The most interesting case for the co-location of related traits in durum wheat, barley and rice is shown in **Fig. 5**. In durum 4A, a locus associated with a QTL for chlorophyll content was mapped 12.5 cM proximal to wg232 locus. This locus was associated with carbon isotope discrimination on durum 4B and closely mapped on barley 4H to the locus cdo795. This locus in turn is associated with osmotic potential in barley and osmotic potential and leaf rolling in rice on chromosome 3. The locus RZ403 on rice chromosome 3 is closely linked to cdo795/RG96 and is associated with a QTL for cell membrane stability on the same chromosome.

These results were expected because these markers are associated with the most significant QTLs in durum wheat (Diab 2003), barley (Diab *et al.* 2004) and rice (**Table 1**). The correlation between chlorophyll content and carbon isotope discrimination has been reported in durum wheat (Merah *et al.* 2000; Villegas *et al.* 2000; Royo *et al.* 2002; Tambussi *et al.* 2002), bread wheat (Richards and Condon 1993), and in barley (Acevedo 1993). These two traits are components of drought tolerance and strongly affect the osmotic potential status of plants, which in turn is responsible for cell membrane stability.

This study utilizes a comparative analysis of durum wheat, barley and rice based on linkage maps with the goal of linking the complex wheat genome to simpler diploid species such as barley and rice that serve as references. However, more detailed comparisons are needed to verify conserved regions associated with drought tolerance and to differentiate the various mechanisms of drought response. The linkage maps with ESTs developed for rice (Harushima *et al.* 1998) and for barely and durum wheat (Diab 2003, 2004) enabled the use of *in silico* sequence comparison of ESTs to align portions of the genomes. Complete sequencing of the rice genome will permit a comprehensive comparison with higher ordering precision that physical and sequence maps will facilitate in the improvement of comparative maps.

Examination of the comparative maps constructed for durum wheat, barley and rice reveals the conservation of several genomic intervals (**Figs. 1-6**), which contain genomic regions and genes associated with drought tolerance. Markers flanking these intervals may provide a starting point for the characterization and positional cloning of these genes in the smaller and less complex genome (e.g. rice). Undoubtedly, as the resolution of the comparative map of durum wheat, barley and rice increases, additional orthologous loci will be identified. Such information could then be applied to all related plant species such as pearl, foxtail and finger millets, which are important for the agriculture of developing countries but are relatively under-resourced in terms of research budgets.

Comparative genomics is very much dependent on continued developments in bioinformatics. Good databases are needed to manage the vast amount of data produced by the sequencing and EST projects and the genetic and physical maps as well as phenotypic data. The establishment of data management systems needs to be paralleled by the development of efficient visualization and data analysis tools so that variation in gene sequence and gene expression can be correlated with variation in key traits. This will allow scientists in all areas of science to benefit from the wealth of information that is available.

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 Relations*, Academic Press, New York, pp 399-417

Alfenito MR, Souer E, Goodman CD, Buell R, Mol J, Koes R, Walbot V (1998) Functional complementation of anthocyanin sequestration in the vacuole by widely divergent glutathione *S*-transferases. *Plant Cell* **10**, 1135-1149

Ali ML, Pathan MS, Zhang JG, Sarkarung BS, Nguyen HT (2000) Mapping QTLs for root traits in a recombinant inbred population from two *indica* ecotypes in rice. *Theoretical and Applied Genetics* **101**, 756-766

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389-3402

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

Capell T, Escobar C, Liu H, Burtin D, Lepri O, Christou P (1998) Over expression of the oat arginine decarboxylase cDNA in transgenic rice (*Oryza sativa* L.) affects normal development patterns *in vitro* and results in putrescine accumulation in transgenic plants. *Theoretical and Applied Genetics* **97**, 246-254

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* **138**, 1251-1274

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. *Theoretical and Applied Genetics* **90**, 969-981

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeolgous group 7 chromosomes. *Theoretical and Applied Genetics* **78**, 495-504

Conley TR, Peng HP, Shih MC (1999) Mutations affecting induction of glycolytic and fermentative genes during germination and environmental stresses in *Arabidopsis*. *Plant Physiology* **119**, 599-607

Devos KM, Gale MD (1997) Comparative genetics in the grasses. *Plant Molecular Biology* **35**, 3-15

Devos KM, Gale MD (1993) Extended genetic map of the homoeologous group 3 chromosomes of wheat, rye and barley. *Theoretical and Applied Genetics* **85**, 649-652

Devos KM, Millan T, Gale MD (1993) Comparative RFLP maps of the homoeologous group-2 chromosome of wheat, rye and barley. *Theoretical and Applied Genetics* **85**, 784-792

Diab AA, Béatrice TM, Dominique T, Neslihan Z O, David B, Mark ES (2004) Identification of drought-inducible genes and differentially Expressed Sequence Tags in Barley. *Theoretical and Applied Genetics* **109**, 1417-1425

Diab AA (2003) Genomic analysis of drought tolerance in wheat and barley. PhD Thesis, Cornell University, Ithaca, New York, USA

Dolferus R, Ellis M, de Bruxelles GL, Frevaskis B, Hoeren F, Dennis ES, Peacock WJ (1997) Strategies of gene action in *Arabidopsis* during hypoxia. *Annals of Botany* **97**, 21-31

Gai XW, Lal S, Xing LQ, Brendel V, Walbot V (2000) Gene discovery using the maize genome database ZmDB. *Nucleic Acids Research* **28**, 94-96

Hansen L, Wettstein-Knowles VP (1991) The barley AcII- and AcI3- encoding acyle carrier proteins I and II are located on different chromosomes. *Molecular and General Genetics* **229**, 467-478

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Saaki T (1998) A high-density rice genetic linkage map with 2275 markers using single F_2 population. *Genetics* **148**, 479-494

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 Physiology* **101**, 833-837

Kato-Noguchi H (2000) Osmotic stress increases alcohol dehydrogenase activity in maize seedlings. *Biologia Plantarum* **43**, 621-624

Kleines M, Ralph-Cyrus E, Maria-Jesus R, Anne-Sophie B, Francesco S, Dorothea B, Max P (1999) Isolation and expression analysis of two stress-responsive sucrose-synthase genes from the resurrection plant craterostigma plantagineum (Hochst.). *Planta* **209**, 13-24

Li Z, Shen LS, Courtois B, Lafitte R (1999) Development of near isogenic introgression line (NIIL) sets for QTLs associated with drought tolerance in rice. Workshop on Molecular Approaches for the Genetic Improvement of Cereals for Stable Production in Water-Limited, 21-25 June, 1999. CIMMYT, El Batan, Mexico, pp 103-107

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC (1996) Locating QTLs for osmotic adjustment and dehydration tolerance in rice. *Journal of Experimental Botany* **47**, 1427-1436

McCouch SR, Doerge BW (1995) QTL mapping in rice. *Trends in Genetics* **11**, 482-487

Mekhedov S, de Ilarduya OM, Ohlrogge J (2000) Toward a functional catalogue of the plant genome. A survey of genes for lipid biosynthesis. *Plant Physiology* **122**, 389-401

Merah O, Monneveux P, Deléens E (2001) Relationships between flag leaf carbon isotope discrimination and several morpho-physiological traits in durum wheat genotypes under Mediterranean conditions. *Environmental and Experimental Botany* **45**, 63-71

Moore G, Devos KM, Wang Z, Gale MD (1995) Cereal genome evolution - Grasses, line up and form a circle. *Current Biology* **5**, 737-739

Morgan JM, Tan MK (1996) Chromosomal location of a wheat osmoregulation gene using RFLP analysis. *Australian Journal of Plant Physiology* **23**, 803-806

Nachit MM, Ketata H (1991) Selection of morpho-physiological traits for multiple abiotic stresses resistance in durum wheat (*Triticum turgidum* L. var. Durum). In: Acevedo E, Conesa AP, Monneveux Ph, Srivastava JP (Eds) *Physiology - Breeding of Winter Cereals for Stressed Mediterranean Environments*, INRA, Paris, pp 391-400

Ozturk NZ, Talame V, Michalowski CB, Gozukirmizi N, Tuberosa R, Bohnert HJ (2002) Monitoring large-scale changed in transcript abundance in drought- and salty-stressed barley. *Plant Molecular Biology* **48**, 551-573

Pichersky E, Gang DR (2000) Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends in Plant Science* **5**, 439-445

Plaisance KL, Gronwald JW (1999) Enhanced catalytic constant for glutathione *S*-transferase (atrazine) activity in an atrazine-resistant *Abutilon theophrasti* biotype. *Pesticide Biochemistry and Physiology* **63**, 34-49

Price AH, Tomos AD, Virk DS (1997) Genetic dissection of root growth in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **95**, 132-142

Price AH, Townend J, Jones MP, Audebert A, Courtois B (2002) Mapping QTLs associated with drought avoidance in upland rice grown in the Philippines and West Africa. *Plant Molecular Biology* **48**, 683-695

Q, X, Stam P, Lindhout P (1996) Comparison and integration of four barley genetic maps. *Genome* **39**, 379 394

Ray JD, Yu LX, McCouch SR, Champoux MC, Wang G, Nguyen HT (1996) Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **42**, 627-636

Redona ED, Mackill DJ (1996) Mapping quantitative trait loci for seedling vigor in rice using RFLPs. *Theoretical and Applied Genetics* **92**, 395-402

Rekika D, Nachit MM, Araus JL, Monneveux P (1998) Effects of water deficit on photosynthetic capacity and osmotic adjustment in tetraploid wheats. *Photosynthetica* **35**, 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. *Australian Journal of Plant Physiology* **21**, 717-730

Richards RA, Condon AG (1993) Challenges ahead in using carbon isotope discrimination in plant breeding programs. In: Ehleringer JR, Hall AE, Farquhar GD (Eds) *Stable Isotopes and Plant Carbon/Water Relations*, Academic Press, New York, pp 451-462

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. *Australian Journal of Agricultural Research* **53**, 561-569

Saghai Maroof MA, Yang G, Biyashev RM, Maughan PJ, Zhang Q (1996) Analysis of the barley and rice genomes by comparative RFLP linkage mapping. *Theoretical and Applied Genetics* **92**, 541-551

Sanchez de la Hoz P, Vicente-Carbajosa J, Mena M, Carbonero P (1992) Homologous sucrose synthase genes in barley (*Hordeum vulgare*) are located in chromosomes 7H (syn 1) and 2H. Evidence for a gene translocation. *FEBS Letters* **310**, 46-50

Sherman JD, Fenwick AL, Namuth DM, Lapitan NLV (1995) A barley RFLP map: alignment of three barley maps and caparisons to Gramineae species. *Theoretical and Applied Genetics* **91**, 681-690

Smit A (1999) RepeatMasker. University of Washington, Seattle, WA. Available online: http://www.phrap.org.

Snape JW, Laurie DA (1996) Comparative mapping and its use for the genetic analysis of agronomic characters in wheat. *Euphytica* **89**, 27-31

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. *Functional Plant Biology* **29**, 35-44

Tarchini R, Biddle P, Wineland R, Tingey S, Rafalski A (2000) The complete sequence of 340 kb of DNA around the rice Adh1-Adh2 region reveals interrupted colinearity with maize chromosome 4. *Plant Cell* **12**, 381-391

Teulat B, Borries C, This D (2001) New QTLs identified for plant water status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. *Theoretical and Applied Genetics* **103**, 161-170

Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A (1998) Several QTLs involved in osmotic adjustment trait variation in barley (*Hordeum vulgare L.*). *Theoretical and Applied Genetics* **96**, 688-698

Tripathy JN, Zhang J, Robin S, Nguyen ThT, Nguyen HT (2000) QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theoretical and Applied Genetics* **100**, 1197-1202

van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995) Molecular genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. *Genome* **38**, 45-59

van Deynze AE, Nelson JC, Yglesias ES, Harrington SE, Braga DP, McCouch SR, Sorrells ME (1995) Comparative mapping in grasses. Wheat relationships. *Molecular and General Genetics* **248**, 744-754

van Deynze AE, Nelson JC, O'Donoughue LS, Ahn SN, Siripoonwiwat W, Harrington SE, Yglesias ES, Braga DP, McCouch SR, Sorrells ME (1995b) Comparative mapping in grasses. Oat relationships. *Molecular and General Genetics* **249**, 349-356

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. *Australian Journal of Agricultural Research* **51**, 891-901

Whittaker A, Bochicchio A, Vazzana C, Lindsey G, Farrant J (2001) Changes in leaf hexokinase activity and metabolite levels in response to drying in the desiccation-tolerant species *Sporobolus stapfianus* and *Xerophyta viscosa*. *Journal of Experimental Botany* **52**, 961-969

Wilson WA, Harrington SE, Woodman WL, Lee M, Sorrells ME, McCouch SR (1999) Inferences on the genome structure of progenitor maize through comparative analysis of rice, maize and the domesticated Panicoids. *Genetics* **153**, 453-473

Zhang J, Zheng HG, Aarti A, Pantuwan G, Nguyen TT, Tripathy JN, Sarial AK, Robin S, Babu RC, Nguyen BD, Sarkarung S, Blum A, Nguyen HT (2001) Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. *Theoretical and Applied Genetics* **103**, 19-29