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Chromosome regions and stress-related sequences involved in resistance to abiotic stress in *Triticeae*

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Abstract

Drought, low temperature and salinity are the most important abiotic stress factors limiting crop productivity. A genomic map of major loci and QTLs affecting stress tolerance in *Triticeae* identified the crucial role of the group 5 chromosomes, where the highest concentration of QTLs and major loci controlling plant's adaptation to the environment (heading date, frost and salt tolerance) has been found. In addition, a conserved region with a major role in drought tolerance has been localized to the group 7 chromosomes. Extensive molecular biological studies have led to the cloning of many stress-related genes and responsive elements. The expression of some stress-related genes was shown to be linked to stress-tolerant QTLs, suggesting that these genes may represent the molecular basis of stress tolerance. The development of suitable genetic tools will allow the role of stress-related sequences and their relationship with stress-tolerant loci to be established in the near future.

Introduction

Physical stresses, particularly drought, low temperature and salinity, place major limits on cereal productivity. Crop species belonging to the tribe *Triticeae* represent world-wide the main foodstuff sources for men and animals; they are cultivated from the northern countries up to the margins of the deserts. Such a great diffusion already suggests that the *Triticeae* genomes should contain genes for wide environmental adaptability and good stress resistance. From the breeding point of view, stress tolerance can be described as the ability to maintain a constantly high yield, regardless of any environmental adversity (a concept known as yield stability). To ensure a high yield stability the ideal genotype should carry favourable alleles at many, possibly all, stress resistance loci. The identification of the genetic components of stress tolerance is, therefore, a requirement to ensure further breeding progress since the traditional selection process has met only limited success due to genotype × environment interactions (Cattivelli *et al.*, 1994). The recent advances in the genetic and molecular understanding of stress responses have led to the identification of a great number of single loci, quantitative trait loci (QTLs) and genes related to stress tolerance. The new knowledge about loci and genes involved in stress resistance is expected to move the selection from phenotype to genotype (marker-assisted selection), thereby reducing the negative effects of the environment and increasing the chances of genetic improvements.

Chromosome regions involved in abiotic stress tolerance

Major loci controlling plant adaptation to seasonal changes

Plant growth habit and heading date are the basic traits involved in the adaptation of cereals to environments since they allow the synchronization of the plant life

cycle with seasonal changes. A number of well defined loci is known to control the plant response to seasonal changes. The genetic factors determining the flowering time can be divided, according to their interactions with environmental signals, into: photoperiod-responsive genes, vernalization-responsive genes, and 'earliness per se' genes largely independent of both day-length and low temperature.

The genetics of earliness per se has been extensively studied in spring barley where flowering does not require vernalization. Traditional genetic linkage studies identified several major genes called *Ea* or *Eam* (early maturity) in barley (Hockett and Nilan, 1985; Gallagher *et al.*, 1991), and *Eps* (earliness per se) in wheat (Worland, 1996). Five *Eam* loci have been mapped by linkage analysis on chromosomes 1H, 2H, 3H, 4H and 6H (positions given in Figure 1) (Franckowiak, 1997). Laurie *et al.* (1995) found thirteen genes (five major genes and eight QTLs) regulating flowering time in a winter × spring barley cross, and among them nine (the *denso* dwarfing gene on chromosome 3H and eight QTLs) were not specifically dependent on photoperiod or vernalization. Photoperiod-responsive loci are known in wheat and barley as *Ppd*. In barley *Ppd-H1*, located on the short arm of chromosome 2H (Laurie *et al.*, 1994), co-maps with and most likely corresponds to the *Ea* (*Eam1*) locus responsible for earliness per se (Figure 1) (Laurie, 1997). A second photoperiod-responsive gene, *Ppd-H2*, has been mapped onto barley chromosome 1H (Laurie *et al.*, 1995). Comparative mapping also shows that *Ppd-H1* is located in a similar map position to the *Ppd* genes of wheat (*Ppd-A1, Ppd-B1* and *Ppd-D1* on the short-arm of chromosomes 2A, 2B and 2D, respectively (Laurie *et al.*, 1994; Snape *et al.*, 1996; McIntosh *et al.*, 1998).

Cereals showing a vernalization response are delayed in flowering unless exposed to low temperatures. Genetic analysis suggests that a relatively small number of conserved genes control the vernalization response (Laurie, 1997; Dubcovsky *et al.*, 1998). In barley, winter habit depends on the presence of the dominant allele at locus *Vrn-H2* (formerly *Sh*) and of the recessive alleles at the loci *Vrn-H1* (*Sh2*) and *Vrn-H3* (*Sh3*). All the other allele combinations among these three genes are found in spring or facultative genotypes (Cattivelli *et al.*, 1994). The loci *Vrn-H2, Vrn-H1* and *Vrn-H3* are located on the long arm of chromosomes 4H, 5H and 1H respectively (Figure 1) (Laurie *et al.*, 1995; McIntosh *et al.*, 1998). Five vernalization-responsive genes (*Vrn1–5*) have been described in wheat (Pugsley, 1973). The most important region for vernalization response is represented by an orthologous position on the long arm of chromosomes 5A, 5B and 5D, carrying the loci *Vrn-A1* (formerly *Vrn1*), *Vrn-B1* (*Vrn2* and *Vrn4*) and *Vrn-D1* (*Vrn3*) (Galiba *et al.*, 1995; Snape *et al.*, 1997). A comparison of a common set of RFLP markers suggests that this region corresponds to the *Vrn-H1* locus of barley (Laurie *et al.*, 1997) and to the *Vrn-R1* (formerly *Sp1*) vernalization response locus of rye (Figure 1) (Plaschke *et al.*, 1993). *Vrn5* had been assigned to the short arm of chromosome 7B (Figure 1) (Law, 1966), in a position not orthologous with other known *Vrn* genes. Similarly also *Vrn-H2* and *Vrn-H3* loci were mapped in genomic regions apparently lacking *Triticum* orthologous loci. Nevertheless, Dubcovsky *et al.* (1998) showed a translocation from the long arm of the homoeologous group 4 to the long arm of homoeologous group 5 where a second *Vrn* gene of *Triticum monococcum*, *Vrn-A^{m}2*, orthologous to *Vrn-H2* (chromosome 4H) was mapped. Curiously, the *Vrn-A2* gene has not yet been detected in bread wheat (Dubcovsky *et al.*, 1998).

The fundamental role of *Vrn, Ppd* and *Ea* genes in determining the heading date was also confirmed by QTL analysis. In the diploid barley more than 80 QTLs for heading date have been mapped up to now in different crosses (http://www.css.orst.edu/barley/nabgmp/qtlsum.htm), with QTLs concentrated on chromosomes 2H (mostly: 19), 7H (17) and 5H (13). Often heading date QTLs map to locations corresponding to previously known *Vrn, Ppd* or *Ea* genes.

Loci controlling stress resistance

Besides vernalization requirement, overwintering genotypes also need to display a frost-tolerant phenotype. Frost tolerance is recognized as a complex quantitative character. A genetic analysis based on wheat chromosome substitution lines showed that loci with a major effect on frost tolerance are carried by chromosomes 5A and 5D (Sutka and Snape, 1989). Thus, when the 5A or 5D chromosome of the frost-sensitive variety Chinese Spring was replaced by the corresponding chromosome of the frost-resistant variety Cheyenne, the frost tolerance of Chinese Spring was greatly increased (Sutka and Snape, 1989). RFLP analysis of chromosome 5A of wheat showed that vernalization requirement and frost resistance are controlled by two different, but tightly linked loci (*Vrn-A1* and *Fr1* respectively) (Figure 1) (Galiba *et al.*,

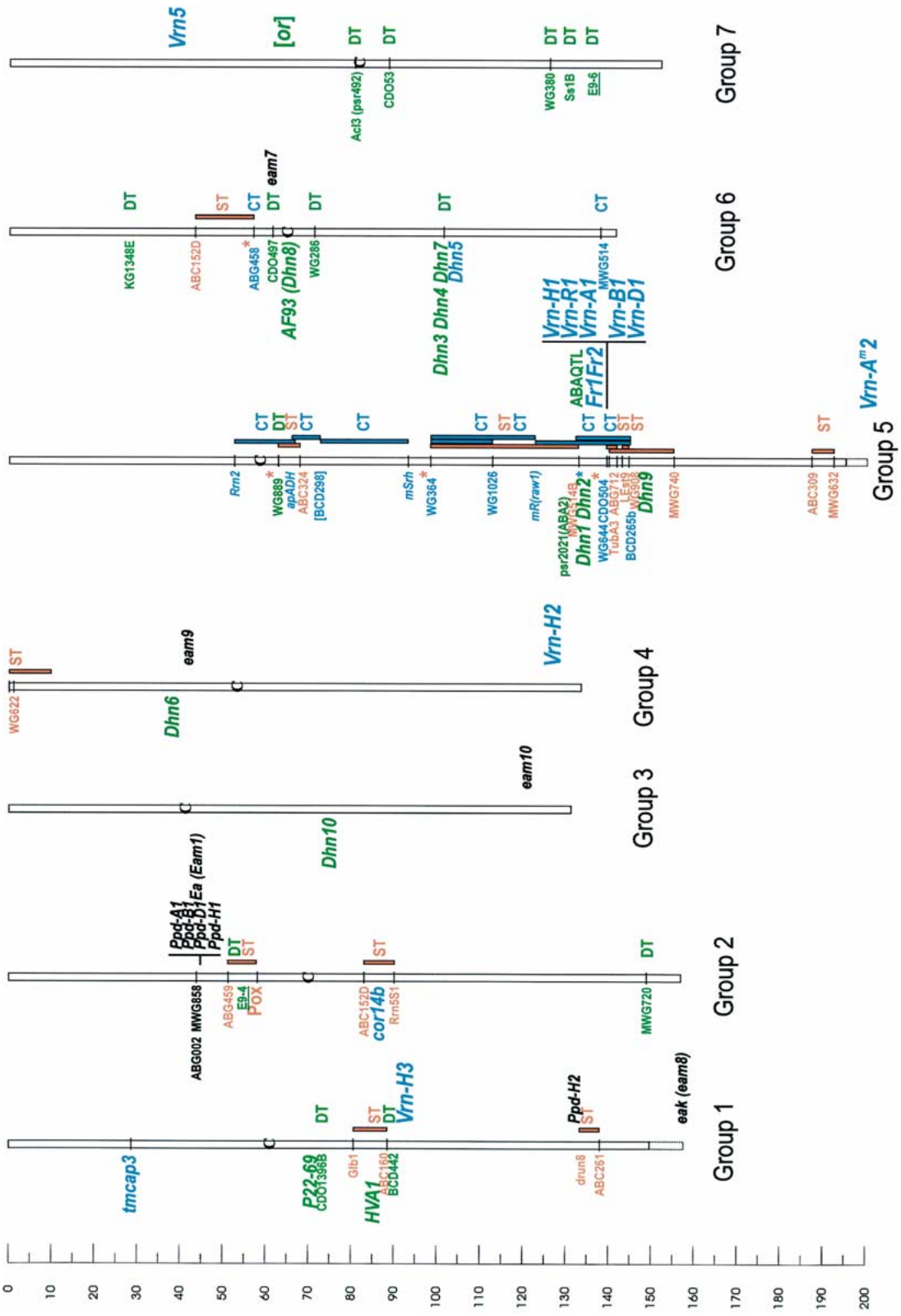
1995; Sutka *et al.* 1999). Because of its large effect on frost resistance, molecular assisted selection for the *Vrn-A1-Fr1* 5A chromosome interval has also been proposed as a tool to improve cold hardiness of wheat cultivars (Storlie *et al.*, 1998). A second frost tolerance locus, *Fr2*, orthologous to *Fr1*, has been recently mapped on wheat chromosome 5D (Snape *et al.*, 1997).

In barley QTLs controlling traits associated with winter-hardiness, such as field winter survival and crown fructan content, were mapped in the Dicktoo × Morex (winter × spring) cross only on the long arm of chromosome 5H (Figure 1) (Hayes *et al.*, 1993; Pan *et al.*, 1994). The authors found evidence for a multilocus cluster of linked QTLs in this region rather than a single QTL with pleiotropic effects. No other genomic regions exceeded the threshold of significance. Nevertheless, a more complex situation was found in the cross Arda × Opale (winter × winter) where nine freezing tolerance QTLs were detected after a screening conducted in a controlled environment (Tuberosa *et al.*, 1997). QTLs were mapped on chromosomes 2H, 3H, 6H and 5H (Figure 1). Not all the QTLs are indicated in Figure 1 because of their linkage with markers neither present in other maps, nor linked to anchor loci. Notably the only QTL on chromosome 5H resided in the vicinity of the *Dhn1* locus, as the field survival QTL revealed by Hayes *et al.* (1993) and Pan *et al.* (1994).

Drought tolerance is an extremely complex trait and many morphological and physiological characters are known to be involved in tolerance/susceptibility, including the presence of awns, tillering, root development, leaf water potential, relative water content (RWC), osmotic adjustment (OA), waxiness, accumulation of osmolytes and ABA. The genetic bases of the relationship between RWC and growth parameters (number of leaves and total biomass) under drought conditions have been dissected in barley by Teulat *et al.* (1997). The centromeric region and the long arm of chromosome 7H were involved in the control of both RWC and leaf number, explaining the negative genetic correlation found between the two traits under water stress; a smaller area of mature leaves implies a lower water loss by transpiration at equal stomatal density. Other QTLs for single traits (either RWC or leaf number) were found on the long arm of 1H, in two regions of 6H, and close to the centromere of 5H (Figure 1). A great importance was given also to the relationship between OA and drought tolerance (Blum, 1989). OA is defined as a decrease of osmotic potential within the cell due to active solute accumulation during a condition of declining leaf water potential. At low soil moisture, osmotic adjustment maintains cell turgor. OA in wheat is conditioned by a major locus designed or located on the short arm of chromosome 7A affecting mainly potassium accumulation (Morgan and Tan, 1996). In a different report, up to ten QTLs for OA were identified in barley (Teulat *et al.*, 1998). Considering all the traits evaluated, the most important genomic regions for drought tolerance were those on chromosomes 7H (centromere) and 6H (long arm) (Figure 1). An increased tissue ABA concentration is thought to play a key role in drought response. A major QTL affecting drought-induced ABA accumulation was mapped on the long arm of chromosome 5A of wheat in the vicinity of the locus controlling frost resistance and tightly linked to the *Dhn1/Dhn2* locus (Figure 1), suggesting a genetic linkage between ABA accumulation and stress tolerance (Quarrie *et al.*, 1994).

An integrated measure of the plant's ability to produce dry matter under water-limiting conditions can be obtained from δ^{13} carbon isotope discrimination which asses water use efficiency (WUE). In C3 plants δ^{13} carbon isotope discrimination is an indicator of the intracellular-to-atmospheric partial pressure of CO_2 (P_i/P_a) and, hence, of the WUE (Farquhar and Richardson, 1984). When δ^{13} discrimination was employed to compare the WUE of wheat, barley and wheat/barley disomic chromosomal addition lines, the results showed barley to possess higher WUE, and identified genetic factors controlling barley WUE on chromosome 4H (Handley *et al.*, 1994). By analysing D-genome substitution lines, Gorny (1999) found that chromosome 7D also positively affects WUE.

Salt tolerance in cereals is known to be associated with the control of shoot Na^+ content; tolerant lines have more efficient systems to exclude sodium from their shoots. A single locus (*Kna1*) localized on chromosome 4D of wheat was shown to control K^+/Na^+ discrimination and, indeed, in a saline environment, bread wheat (genomes AABBDD) accumulates less Na^+ and more K^+ than durum wheat (genomes AABB) (Dubcovsky *et al.*, 1996). Loci involved in salt tolerance have been identified on chromosomes 4H and 5H of *H. vulgare* and $1H^{ch}$, $4H^{ch}$ and $5H^{ch}$ of *H. chilense* (Forster *et al.*, 1990). The gene pool of wild relatives may represent an interesting source of new loci for salt tolerance. A number of QTLs affecting salt tolerance were detected on all chromosomes in a cross between *H. vulgare* subsp.


Group 1
Group 2
Group 3
Group 4
Group 5
Group 6
Group 7
tmcap3
P22-69
HVA1
Vrn-H3
Ppd-H2
eak (eam8)
Ppd-A1
Ppd-B1
Ppd-D1
Ea (Eam1)
Ppd-H1
cor14b
Dhn10
eam10
Dhn6
eam9
Vrn-H2
Dhn1
Dhn2*
Dhn9
ABAQTL
Fr1Fr2
Vrn-A^m2
Dhn3
Dhn4
Dhn7
Dhn5
AF93 (Dhn8)
eam7
Vrn-H1
Vrn-R1
Vrn-A1
Vrn-B1
Vrn-D1
Vrn5
[or]
0
10
20
30
40
50
60
70
80
90
100
110
120
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160
170
180
190
200

Figure 1. Summary of abiotic stress tolerance QTLs mapped on the homoeologous *Triticeae* chromosome groups. Summary map, chromosome length and map comparisons are based on the barley consensus map of Qi *et al.* (1996). Chromosome length scale (Kosambi cM) is reported on the left side. Group 1 and group 5 chromosome long arms are longer than originals (dashed) to allow mapping of telomeric loci. Centromere (C) positions have been calculated from the consensus map of Langridge *et al.* (1995). On the left side of the chromosomes small characters indicate RFLP and RAPD (underlined) marker loci; italics in larger fonts show the map position of known stress-related genes. Loci belonging to the consensus map are indicated by a line on the chromosome bars; the positions of all other loci have been calculated by bridging maps and weighting distances between common markers. Square brackets indicate loci whose map location is uncertain or contrasting with known locations of anchor RFLP loci reported on consensus maps. Round parentheses indicate synonymous markers and loci. Boxes on the right side of chromosomes indicate map intervals (length-weighted) where QTLs have been mapped by interval mapping procedures. Capitals indicate QTL peaks and most significant marker loci associated with QTLs: CT, cold tolerance; DT, drought tolerance; ST, salt tolerance; ABAQTL, ABA accumulation QTL. Earliness per se (*eam*), photoperiod response loci (*Ppd*), vernalization-responsive (*Vrn*), and frost tolerance (*Fr*) loci are reported. Different colours indicate the stress specificity of markers, loci, stress-related genes and QTLs: blue, cold; green, drought; orange, salt; black, none. Markers carrying an asterisk of a different colour are 'multiple stress' markers tagging QTLs for tolerance to different stresses.

spontaneum and *H. vulgare*, although several QTL clusters were present on chromosomes 1H, 4H, 6H and 7H (Ellis *et al.*, 1997); QTL positions are not reported in Figure 1 because of the lack of anchor markers for certain localisation on consensus maps. The analysis of wheat cytogenetic stocks has demonstrated that the homoeologous group 5 carries loci involved in the response to salt stress in hydroponic conditions (Koebner *et al.*, 1996). Chromosone 5E coming from the wild species *Thinopyrum bessarabicum* or *Lophopyrum* (*Thinopyrum*) *elongatum* was shown to increase wheat salt tolerance (Kasai *et al.*, 1998). QTLs controlling salt tolerance at germination (chromosomes 1H, 4H, 5H and 6H) and at the seedling stage (chromosomes 1H, 2H, 5H and 6H) have been mapped in cultivated barley (Figure 1) (Mano and Takeda, 1997). Notably, the two QTL sets do not overlap, a finding in agreement with previous evidence suggesting that different genetic mechanisms control salt tolerance during germination and plant growth (Mano and Takeda, 1997).

A genomic map of major loci and QTLs affecting abiotic stress tolerance in Triticeae was compiled and shown in Figure 1. Chromosome group 5 has the highest concentration of QTLs and major loci controlling plant adaptation to the environment, particularly those controlling heading date, frost and salt tolerance, whereas a region with a crucial role in drought tolerance is located on chromosome group 7. Although the molecular responses to cold and drought stresses share a common set of genes (see below), the loci describing the genetic bases of cold and drought tolerance are different. Nevertheless, multiple-stress QTLs and linked markers have also been detected, suggesting the existence of common mechanisms for different stresses, or of clusters of genes controlling different stress tolerance processes.

Identification of stress-related sequences

Stress-related genes

The application of cloning techniques to the analysis of the cereal stress response has led to the isolation of a large number of genes whose expression is affected (in most cases up-regulated) by the stress event. According to sequence similarities, expression behaviour and function, the stress-related genes isolated from the *Triticeae* genomes can be divided into several groups as listed in Table 1 (the map position of most stress-related genes is also presented in Figure 1). The precise function of many stress-related gene products still remains unclear, although their expression pattern strongly suggests a connection between their activity and stress tolerance. Adaptation mechanisms are likely to be widely conserved among different plant species. This is evident especially for the LEA (late embryogenesis-abundant) gene family, one of the most common class of genes induced in the vegetative tissues by dehydration, cold, ABA treatment, salt and osmotic stress and which accumulate in the embryos during desiccation. The LEA genes cloned from *Triticeae* have been divided into three classes with respect of their conserved amino acid motifs. The LEA class 1 genes code for highly hydrophilic proteins with a very high content of glycine and charged residues, containing one to four copies of a conserved 20 amino acid repeat, N-terminal (GETWPGGTGGK) and C-terminal (EGIDIDESKF) consensus amino acid motifs (Espelund *et al.*, 1992). LEA class 2 (known as dehydrins) represents the main group of stress-related proteins. Dehydrins are characterized by one or more conserved lysine-rich 15-amino acid sequence (EKKGIMDKIKEKLPG) near the C-terminus and, in most cases, by a stretch of serine residues where phosphorylation may occur. Among the dehydrin family, basic dehydrins are highly expressed during dehydra-

Table 1. Stress-related genes isolated from the Triticeae genomes.

Gene class	Isolated clones	Expression	References
Stress-related genes with unknown function: LEA group 1 (Em-like)	*Em* *B19* gene family *PSP 1015* *CS41*	Expressed during embryo desiccation tolerance and in young seedlings salt- and water-stressed.	Cuming, 1984 Espelund *et al.*, 1992 Hollung *et al.*, 1994 Litts *et al.*, 1987
Stress-related genes with unknown function: LEA group 2 (Dehydrins)	Barley: *dhn 1-13* *af93* Wheat *WCS120* and related genes *WCOR410* *Wsp15, 23* *Rab 15* *Tddhn 15,16, 9.6*	Expressed in vegetative tissues during drought and/or cold stress.	 Choi *et al.*, 1999 Grossi *et al.*, 1995 Houde *et al.*, 1992 Danyluk *et al.*, 1998 Joshi *et al.*, 1992 King *et al.*, 1992 Labhilili *et al.*, 1995
Stress-related genes with unknown function: LEA group 3	*HVA 1* *MA 2005*	Expressed in the aleurone during embryo desiccation tolerance and in vegetative tissues during drought, salt and cold stress.	Hong *et al.*, 1992 Curry *et al.*, 1991
Other stress-related genes with unknown function	*Blt14-Rlt14* gene family	Expressed during cold acclimation in the cortex and in cell layers surrounding vascular bundles.	Cattivelli and Bartels, 1990; Dunn *et al.*, 1990; Grossi *et al.*, 1998
	Blt101 gene family	Expressed during cold acclimation particularly in the crown perivascular layers.	Goddard *et al.*, 1993
	Tacr7	Accumulated during ABA-induced freezing tolerance in immature embryos.	Gana *et al.*, 1997
	AWPM19 (plasma membrane polypeptide)	Expressed during cold acclimation.	Koike *et al.*, 1997
	ScRPS7 (cytoplasmic ribosomal protein S7)	Expressed during cold acclimation.	Berberich *et al.*, 2000
	Blt801 (codes for a RNA binding protein)	Expressed during cold acclimation.	Dunn *et al.*, 1996
	Cor14b, Wcs19	Cold-induced and light-stimulated, chloroplast-localized proteins.	Chauvin *et al.*, 1993; Crosatti *et al.*, 1995
	WESR1-4	Early salt-responsive	Nemoto *et al.*, 1999
Stress-related genes with known function	*PG22-69* (homologous to aldose-reductase)	Expressed during embryo desiccation tolerance.	Bartels *et al.*, 1991
	BADH (betaine aldehyde dehydrogenase)	Expressed during water stress.	Ishitani *et al.*, 1995
	MBM1 (L-isoaspartyl methyltransferase)	Expressed during water stress.	Mudgett and Clarke, 1999
	TaSAMDC (homologous to *S*-adenosylmethionine decarboxylase)	Expressed during osmotic and drought stress.	Li and Chen, 2000
	Bnuc1 (nuclease I)	Expressed during osmotic stress.	Musamoto *et al.*, 1999
	PKABA1 (serine/threonine protein kinase)	Expressed during water stress.	Anderberg and Walker-Simmons, 1992
	Blt63 (elongation factor 1α)	Expressed during cold acclimation.	Dunn *et al.*, 1998
	Blt4 gene family (codes for non specific lipid transfer protein)	Expressed during cold acclimation and drought stress.	Dunn *et al.*, 1996

Cor tmc-ap3 (homologous to amino acid selective channel protein)	Constitutively expressed, enhanced by cold, chloroplast-localized protein.	Baldi *et al.*, 1999
Wcr12 (early light-inducible protein)	Expressed during cold acclimation.	Shimosaka *et al.*, 1999
CHT9, CHT46 (code for chitinase antifreeze proteins)	Expressed during cold acclimation	Yet *et al.*, 2000
WESR 5 (glucose-6-phosphate dehydrogenase)	Expressed during osmotic stress.	Nemoto *et al.*, 1999

tion, but not during cold treatment, while the acidic dehydrins are mainly cold-responsive (Choi *et al.*, 1999, 2000; Zhu *et al.*, 2000). Immunoelectron microscopy revealed that the wheat acidic dehydrin WCOR410 accumulates in the vicinity of the plasma membrane suggesting an involvement of this dehydrin class in membrane cryoprotection (Danyluk *et al.*, 1998). The proteins of LEA class 3 contain a tandem repeat motif of 11 amino acids that may form an amphiphilic α-helix structure (Dure, 1993). Firstly isolated in dormant seeds, they were found to be expressed during embryo desiccation, in water-stressed, ABA, salt- and cold-treated seedlings (Curry *et al.*, 1991; Hong *et al.*, 1992; Sutton *et al.*, 1992).

A number of stress-related sequences, known as *cor* or *blt*, have been found to be up-regulated by low temperature and most likely involved in the acquisition of frost tolerance. Their expression may also be affected by ABA or other environmental factors such as dehydration or light, although *cor* genes activated only at low temperature also exist. A detailed analysis of these stress-related sequences has revealed the existence of multigene families and tissue specific expression. In barley, the *blt14* gene family is composed of at least five members, all post-transcriptionally up-regulated only in response to cold (Dunn *et al.*, 1994; Phillips *et al.*, 1997; Grossi *et al.*, 1998). Each member of the gene family showed a different expression pattern, different threshold induction temperature and genotype-dependent induction kinetics. *In situ* hybridization analysis showed that *blt14* is expressed only in the inner layers of the cortex and in cell layers partly surrounding vascular bundles of cold-acclimated barley (Pearce *et al.*, 1998). To date the function of these genes is still unknown, although a putative signal peptide for the secretory pathway has been identified.

The low temperature response leads to a complete re-arrangement of the cell metabolism and several *cor* genes were found to be involved in amino acid transport or protein synthesis, while others were found to encode membrane proteins. In barley, both the expression of a chloroplastic amino acid selective channel protein (*cor tmc-ap3*) (Baldi *et al.*, 1999) and of the elongation factor-1α (*blt63*) (Dunn *et al.*, 1993) are strongly enhanced during cold treatment. Furthermore, the cold-regulated gene *blt801* was found to encode a protein with RNA binding activity (Dunn *et al.*, 1996). In rye, the low-temperature-induced gene *ScRPS7* showed sequence similarity with the cytoplasmic ribosomal protein (Berberich *et al.*, 2000). The cold-induced wheat gene *tacr7* encodes a protein with a single transmembrane domain and an amino acid bias for leucine (Gana *et al.*, 1997), while the wheat *awmp-19* clone encodes a highly hydrophobic peptide with four membrane-spanning domains. The elevated pI and hydrophobic nature suggest that AWPM-19 is a membrane protein involved in the interaction with negatively charged molecules (Koike *et al.*, 1997). The barley *blt4* gene family revealed homologies with non-specific lipid transfer proteins. Expression of *blt4* was found confined to the epidermis of the leaf and to the epidermis of the older parts of the crown (Pearce *et al.*, 1998). All members of the *blt4* gene family have an extracellular transport consensus signal peptide in the N-terminus suggesting a possible involvement in wax synthesis or secretion (Dunn *et al.*, 1991).

Plant cold acclimation also involves the expression of stress-related genes encoding chloroplast-imported proteins. The first gene isolated with such characteristics was *cor14b* (formerly *pt59*) (Cattivelli and Bartels, 1990; Crosatti *et al.*, 1995). Both *cor14b* and the wheat homologous *wcs19* (Chauvin *et al.*, 1993) are induced only by low temperature and their expression is enhanced after even brief exposure to

light, a condition needed for a complete development of the chloroplast (Crosatti *et al.*, 1999). Plant exposure to cold also promotes the accumulation of early light inducible proteins (ELIP). ELIPs are small proteins also accumulated during greening of etiolated seedlings and localized in the stroma of chloroplasts in the vicinity of D1 protein (Shimosaka *et al.*, 1999).

The accumulation of polypeptides able to interfere with the growth of ice crystals (antifreeze proteins) in the apoplast represents a further component of the cold response. In rye several antifreeze proteins which are highly homologous to pathogenesis-related proteins have been purified and two cold-responsive genes (*CHT9* and *CHT46*) coding for chitinases with antifreeze properties have been cloned (Yet *et al.*, 2000).

Plant cells respond to variations in osmotic conditions by triggering several genes involved in accumulation of osmolytes or in the repairing of damaged cell structures. In barley a gene coding for aldose reductase, the enzyme of the polyol pathway involved in the accumulation of sorbitol, is expressed during embryo development and modulated by ABA during dehydration (Bartels *et al.*, 1991). The genes coding for L-isoaspartyl methyltransferase and betaine aldehyde dehydrogenase (BADH) are up-regulated in wheat plants subjected to high salt or drought conditions (Mudgett and Clarke, 1994; Ishitani *et al.*, 1995; A.M. Mastrangelo, unpublished). L-isoaspartyl methyltransferase is an enzyme involved in the repair of damaged proteins, while BADH is the last enzyme of the pathway leading to glycine betaine, a typical osmolyte accumulated in some plants during stress response. In wheat the gene coding for *S*-adenosylmethionine decarboxylase (*TaSAMDC*), the key enzyme of spermidine and spermine biosynthesis, was also found up-regulated under osmotic stress or drought conditions (Li and Chen, 2000). A wheat gene coding for a protein kinase (*PKABA1*) was also found enhanced during water stress (Anderberg and Walker-Simmons, 1992) suggesting the involvement of phosphorylation events in the transduction pathway controlling the water stress response.

cis-*responsive elements*

The regulation of stress-responsive genes appears to be a very complex mechanism. The study of the promoter regions of these genes can be considered a key step for the comprehension of the molecular mechanisms leading to gene activation. Sequence analysis has revealed that different motifs are required for stress activation (*cis*-acting elements). Each element responds to a specific hormone or environmental stimulus, therefore the combination of different factors confers specificity to the stress-related gene expression. Functional dissection of ABA-inducible gene promoters revealed specific ABA-responsive elements (ABREs) containing the ACGT core element. In cereals different ABREs have been found. The first examples were the wheat *Em1a* ABRE (Marcotte *et al.*, 1989) and *MotifI* ABRE from rice *rab16* (Mundy *et al.*, 1990). Multiple copies of these elements fused to a minimal 35S promoter were able to confer ABA responsiveness to a reporter gene. The promoter of the maize gene *rab17* contains at least five ABREs, but not all of them are active in the same tissue and in the same developmental stage (Busk *et al.*, 1997). This indicates that in many cases the ABRE motif alone is not sufficient for gene activation, but an additional element (often named coupling element or CE) is necessary. A first coupling element (CE1) has been identified in the promoter of the barley gene *HVA22* (Shen and Ho, 1995). This element acts together with a G-box type ABRE in conferring high ABA induction. Another coupling element (CE3) has been identified in the ABA-inducible barley gene *HVA1* (Shen *et al.*, 1996). These two barley coupling elements are not completely interchangeable thus demonstrating their different roles in the control of ABA-induced gene expression.

The promoter of the wheat ABA-inducible *FKBP73* gene, a sequence involved in seed maturation and germination, is characterized by a number of different regulatory sequences, among which there are three G-box ABREs designated as ABRE1, ABRE2 (identical to the wheat *Em1a* ABRE) and ABRE3 (identical to the rice *rab16 MotifI* ABRE). Three CE1-like elements were found proximal to each ABRE. Each CE1-ABRE motif was referred to as an ABA-responsive complex (ABRC). Removal of the two distal ABRCs had little effect, while deletion of the TATA proximal ABRC caused a substantial decrease in promoter activity (Kurek *et al.*, 2000).

Besides ABREs, other *cis*-acting elements are involved in stress responses. In *Arabidopsis* a drought-responsive element (DRE) corresponding to the sequence TACCGACAT has been found in the promoter of several stress-related genes (*lti78/rd29a* and *lti76/rd29b*) (Yamaguchi-Shinozaki and Shinozaki, 1994). The DRE controls the cold and drought induction of *rd29a* in an ABA-independent manner.

The expression of *rd29a* is ABA-independent in the first few hours after dehydration, but becomes ABA-dependent in the later stages of expression. Indeed, an ABRE was also present in the promoter of *rd29a*, suggesting that both ABRE and DRE could interact to control gene expression during dehydration. A motif very similar to DRE (named C-repeat: TGGCCGAC) was responsible for the cold induction of the *Arabidopsis* cold-inducible gene *cor15a* (Baker *et al.*, 1994).

After the identification of DRE/C-repeat elements in *Arabidopsis*, similar responsive elements have been found to control the stress-related expression in many different plant species. DRE-like elements have been found in the promoter regions of drought-inducible genes such as *rab17* in maize (Busk *et al.*, 1997), *wsi18* in rice (Joshee *et al.*, 1998) or *HVA1* in barley (Straub *et al.*, 1994). The DRE core motif, CCGAC, was also present in the promoter sequences of cold-regulated genes from oilseed rape (Jiang *et al.*, 1996), wheat (Ouellet *et al.*, 1998) and barley (Dunn *et al.*, 1998). The promoter of the wheat cold-induced gene *wcs120* contains several putative low temperature responsive elements (LTRE) with the core motif CCGAC. This promoter sequence was active in both monocotyledon and dicotyledon species, suggesting that universal transcription factors responsive to low temperature may be present in all plants (Ouellet *et al.*, 1998). The promoter of the barley cold-induced gene *blt4.9* contains a CCGAC element as well as the related sequence CCGAAA. Nevertheless only the CCGAAA element acted as binding site for a low-mobility nuclear protein complex in electrophoretic mobility shift assay, suggesting that CCGAAA and not CCGAC works as LTRE in barley (Dunn *et al.*, 1998).

Transcription factors

The isolation of the protein factors which specifically interact with *cis*-acting stress-responsive elements is a fundamental step towards the biotechnological improvement of stress tolerance. Most work has been carried out in *Arabidopsis*, although, due to the similarities in the basic stress response mechanisms between model plants and crops, such factors are expected to exist in all species. The ABRE *cis*-acting elements are bZIP type (basic domain/leucine zipper) transcription activators (Yamaguchi-Shinozaki and Shinozaki, 1994). In rice the bZIP protein OSBZ8 was shown to bind to G-box and G-box-like elements, including ABREs. The *OSBZ8* gene is induced by ABA treatment both in embryos and young tissues and its accumulation preceded that of typically ABA-regulated genes (Nakagawa *et al.*, 1996). Abe *et al.* (1997) have recently described MYC- and MYB-type transcription factors also involved in the ABA and desiccation stress responses. A 67-bp DNA fragment of the promoter of the *Arabidopsis* dehydration-responsive gene *rd22*, containing putative recognition sites for the basic helix-loop-helix proteins MYC and MYB, has been shown to be sufficient for dehydration- and ABA-induced gene expression. Both the MYC-related (*rd22BP1*) and MYB-related (*ATMYB2*) transcription factors have been identified and shown to activate transcription of the *rd22* gene. In cereals, putative MYB recognition sites have been found in the wheat *FKBP73* promoter region (Kurek *et al.*, 2000), although no MYB binding has been proved at present.

In *Arabidopsis* a class of transcription factors has been identified based on their ability to bind DRE-LTRE responsive elements. Five DRE/LTRE-binding protein genes (DREB) are known so far (Liu *et al.*, 1998). All these proteins contain the AP2 DNA-binding domain similar to that present in *Arabidopsis* APETALA2 and EREPB (ethylene response element-binding protein). DREBs have been classified into two groups, DREB1 and DREB2, according to their sequence similarities. DREB1A, DREB1B and DREB1C proteins, also described as cold binding factors CBF (Stokinger *et al.*, 1997; Gilmour *et al.*, 1998), are induced by cold stress, while DREB2A and DREB2B are regulated by dehydration (Liu *et al.*, 1998). These findings suggest that the same *cis*-acting element (DRE/LTRE) could be involved in response to different stresses through different *trans*-acting elements (DREB1 and DREB2).

Mutants and transgenic plants

On the basis of sequence homologies or *in vitro* experiments a putative function has been proposed for some of the stress-related genes cloned so far. Nevertheless, evidence of the physiological role of such genes and of their involvement in stress resistance can be found only through a genetic approach based on plant transformation or on the identification of mutants.

One of the most successful strategies to improve stress resistance is based on increased osmolyte (mainly glycinebetaine or proline) concentration through transformation with genes controlling osmolyte biosynthesis (reviewed by Nuccio *et al.*,

1999), while only few results have been obtained by over-expressing stress-related genes. Yeast cells accumulating the type 1 LEA protein Em from wheat exerted an increased resistance to osmotic stress, but not to freezing (Swire-Clark and Marcotte, 1999). The only report on transgenic plants with improved stress tolerance as a consequence of accumulation of LEA proteins concerns HVA1, a type 3 LEA protein originally isolated from the aleurone layers of barley seeds. Transgenic rice and wheat plants characterized by high-level accumulation of the HVA1 protein showed increased tolerance to water deficit and salt stress, as indicated by higher growth rates, delay in the development of the major stress-related symptoms, and faster recovery after stress, than wild-type plants (Xu *et al.*, 1996; Sivamani *et al.*, 2000).

The over-expression of many other stress-related genes did not affect significantly the stress tolerance, although a negative result does not necessarily mean that these genes have no role in stress tolerance since they may represent single components of a complex multigenic response. A way to manipulate the whole, or a large part of the stress response machinery is represented by the utilization of regulatory genes which activate many downstream genes. A pioneering example of this is represented by the development of transgenic *Arabidopsis* plants expressing the DRE-binding protein DREB1 under the control of a stress inducible promoter. Transformed plants showed over-expression of a number of stress-inducible genes and improved tolerance to drought, salt, and freezing stress (Kasuga *et al.*, 1999). Similarly, transgenic rice plants over-expressing the gene *OsCDPK7*, coding for a calcium-dependent protein kinase naturally induced during cold and salt stress, showed enhanced expression of a set of stress-responsive genes and improved stress tolerance (Saijo *et al.*, 2000).

The development of plant mutants with altered stress response is a powerful tool to dissect signal transduction pathways and to understand the role of stress-related genes in plant stress tolerance. In recent years many mutants with a stress-related phenotype have been selected. In *Arabidopsis* mutant genes conferring ABA-deficiency and ABA-insensitivity have been cloned providing new insights into the role of ABA in stress response (Leung and Giraudat, 1998). Frost-resistant as well as frost-susceptible *Arabidopsis* mutants have also been found. The analysis of the different mutants available clearly indicates that stress tolerance is the result of many different components induced during stress acclimation, i.e. accumulation of osmolytes (Xin and Browse, 1998), expression of stress-related genes (Knight *et al.*, 1999), etc. Similar mutants have only rarely been isolated in the *Triticeae*. In barley, ABA-insensitive mutants are known. The first example concerns the identification of a mutant with ABA-insensitive stomata (Raskin and Ladyman, 1988). More recently Molina Cano *et al.* (1999) have isolated a mutant, designed TL43, characterized by a reduced dormancy as it can tolerate a ten-fold increase in ABA, compared to its wild type, before germination is inhibited. When grown in field conditions, TL43 showed a considerable yield reduction due to limited tiller number and spike size. Wheat mutants showing improved thermotolerance (Mullarkey and Jones, 2000), greater affinity for strongly bound water in leaves (Rascio *et al.*, 1999) or improved tolerance to osmotic stress (Rascio *et al.*, 2001) have also been isolated after chemical mutagenesis.

All mutants described above have been selected on the basis of a stress tolerant/susceptible phenotype. Alternatively mutants can also be selected according to their inability to correctly induce specific stress-related genes. It has been found that the cold-induced expression of several *cor* genes (*cor14b, cor tmc-ap3* and *blt14*) was strongly impaired in barley *albino* plants carrying a mutation at the locus a_n, demonstrating the involvement of the chloroplast in the signal transduction pathway leading to the expression of some *cor* genes (Grossi *et al.*, 1998; Baldi *et al.*, 1999; Crosatti *et al.*, 1999). Notably, the albino mutant a_n was completely unable to harden when exposed to low temperature, providing evidence of the relationship among photosynthesis, expression of cold-regulated genes and the development of cold hardening (Grossi *et al.*, 1998).

Associations between QTLs and stress-related genes

The genetic and molecular dissection of stress tolerance has led to the identification of either genomic regions involved in stress tolerance (major loci or QTLs), or DNA sequences known to play a role in molecular stress responses (stress-related genes, *cis*-acting elements and transcription factors). Although QTL analysis and gene cloning have been used to investigate the same stress responses, the relationship between QTLs and stress-related sequences is still far from understood and will remain a challenge for the near future.

The most direct approach is represented by map positional cloning of the genes responsible for the QTL effects. The first plant QTLs, *Brix9-2-5* (increasing sugar content in tomato and coding for an apoplastic invertase) (Fridman *et al.*, 2000) and *Hd-1* (controlling heading date in rice and coding for a zinc finger transcription factor) (Yano *et al.*, 2000) have been recently cloned. These results show that the molecular basis of a QTL can be explained either by a gene directly involved in the biochemical pathway leading to the phenotype or by a transcription factor controlling the expression of many genes. Molecular analysis of the stress response identified a number of genes which are thought to have a role in stress tolerance. Therefore, they were used as 'candidate genes' to search for possible co-segregation with known QTLs. For instance, several pathogen-related genes were shown to co-map with QTLs for disease resistance in wheat. On the long arm of chromosome 7B a cluster of pathogen-related genes (catalase, chitinase, thaumatins and an ion channel regulator) overlapped with a QTL having major effects on leaf rust resistance (Faris *et al.*, 1999). Many stress-related genes have been mapped and some of them have been shown to co-segregate with stress tolerance QTLs (Figure 1). Two *Dhn* loci (*Dhn1/Dhn2* and *Dhn9*) are located in the same region of chromosome group 5 where *Vrn-1* and *Fr1* major loci, cold and salt tolerance QTLs and ABA accumulation QTLs have been mapped. Similarly, the *Dhn* cluster comprising *Dhn3, Dhn4, Dhn5* and *Dhn7* and the locus of *Dhn8/pAF93* on chromosome group 6 are associated with drought tolerance QTLs. Several other drought-induced genes such as *PG22-69* and *HVA1* (chromosome group 1) are associated with QTLs for osmotic adjustment (Teulat *et al.*, 1998). Interestingly, the expression of the LEA gene *HVA1* in transgenic rice was demonstrated to confer tolerance to both water deficit and salt stresses (Xu *et al.*, 1996). As a last observation, the alcohol dehydrogenase (an enzyme of anoxic metabolism) locus *apADH* maps to the colinear region of the *Sub1* rice major gene responsible for flooding tolerance Hayes *et al.*, 1993; Xu and Mackill, 1996; Dubcovsky *et al.*, 1998). Nevertheless, only a few studies provide genetic evidence that the stress tolerance effect explained by a given QTL can be attributed to a co-mapping stress-related gene. In cowpea the accumulation of the 35 kDa dehydrin was found to be involved in chilling tolerance during seedling emergence. Allelic differences in the coding region of the dehydrin structural gene map to the same position as the dehydrin protein presence/absence trait which in turn is associated with chilling tolerance/susceptibility (Ismail *et al.*, 1999). These results also demonstrate that allelic variations in a stress-related gene can significantly alter plant stress tolerance ability. Allelic variations, potentially related to environmental adaptation, were also found for the barley cold-regulated COR14b protein in a collection of *H. vulgare* ssp. *spontaneum* accessions (Crosatti *et al.*, 1996).

Besides the examples described above, there are stress-related genes located clearly outside any stress tolerance QTLs. Although the barley cold-regulated genes *cor14b* and *cor tmc-ap3* (chromosomes 2H and 1H, respectively) are expressed at higher levels in frost-resistant than in susceptible cultivars, none of them maps on chromosome 5H where almost all cold tolerance QTLs have been localized (Crosatti *et al.*, 1996; Baldi *et al.*, 1999; Mastrangelo *et al.*, 2000). These results raise the possibility that the molecular basis of a QTL for stress tolerance could be explained by a regulatory gene able to control the expression of many stress-related genes. Vagujfalvi *et al.* (2000) have indeed shown that in wheat the expression of *cor14b* is controlled by two loci (*Rcg1* and *Rcg2*) located in the *Vrn-1A/Fr1* region of chromosome 5A, demonstrating the genetic linkage between the expression of cold-regulated genes and the genomic region carrying frost resistance loci. Similarly, Fowler *et al.* (1996) have reported that loci of the *Vrn-1A/Fr1* region explain the different expression levels of *wcs120* found in resistant and susceptible wheat and rye cultivars. After the discovery of the *Arabidopsis* DRE-CBF transcription factors it has been suggested that the cereal loci controlling stress tolerance (i.e. the *Fr1* locus) could represent the DRE-CBF cereal homologous genes (Sarhan and Danyluk, 1998). However, so far direct evidence for this hypothesis is still lacking.

Conservation of stress-related sequences among plant genomes

QTL mapping, gene cloning, ESTs (Expressed Sequence Tags) and genome sequencing projects have led to a vast body of genetic information in public databases supplying the scientific community with powerful tools for comparative genomics (Gai *et al.*, 2000; Mekhedov *et al.*, 2000). The integration of genetic information from related species can lead to the identification of highly conserved sequences and/or regulatory mechanisms by which it is possible to pre-

dict function and location of genes in different organisms that have been traditionally studied separately. The analysis of the stress response of different plant species can be carried out by sequence comparison of stress-related genes and of *cis*- or *trans*-acting elements or by looking for conserved positions of stress tolerance loci among related genomes.

The analysis of stress-related gene sequences from a number of evolutionarily distant plants reveals that the molecular response to stress is conserved, to some extent, among different species. To give a general estimate of the homology level between stress-related genes isolated from the *Triticeae* genomes and those isolated from other species a search in the sequence databases was performed to identify all wheat, barley or rye entries described as induced by drought and/or cold stress. Sequences where chosen to avoid duplications and these entries were used for a BLASTN search against the genome of the two model plants rice and *Arabidopsis*. A BLASTN score higher or equal to 50 and 80 was used to declare homology with *Arabidopsis* and rice sequences respectively. The different stringency adopted for testing *Arabidopsis* and rice reflected the closer evolutionary relationship of rice with the *Triticeae* group, which would predict a higher similarity for longer stretches of nucleotides. A first BLASTN analysis was carried out with about 40 different LEA entries. The results indicated that 88% of the *Triticeae* stress-related LEA sequences found a corresponding match in rice and 60% in *Arabidopsis*. Similarities are not restricted to the LEA protein gene sequences, but they also concern the mechanisms controlling gene expression. Promoter elements of the wheat gene *Em* were shown to be active even in a distantly related species such as moss. A mutational analysis indicated that the mechanism of gene regulation is exactly conserved between cereals and the moss *Physcomitrella patens* (Knight *et al.*, 1995). Expression of LEA protein genes is also a component of the cold stress response and it has been suggested that the regulatory mechanisms leading to cold-induced LEA accumulation are also conserved in both monocotyledonous and dicotyledonous species. The promoter region of the wheat cold-induced gene *wcs120* showed a low-temperature-inducible activity in the *Triticeae*, rice, Cruciferae, *Leguminosae* and *Cucurbitaceae*, but not in *Solanaceae* (Ouellet *et al.*, 1998).

Besides dehydrins, the molecular dissection of the cold response in barley and wheat has led to the isolation of many other genes, most of which are characterized by a low temperature specific expression (no drought or ABA induction). For instance, the expression of *cor14b*, a typical *cor* gene expressed only at low temperature, was found to be independent from the accumulation of dehydrin mRNAs suggesting that the two gene classes are controlled by different signal transduction pathways (Crosatti *et al.*, 1998). A search in the databases revealed about 25 wheat, barley or rye entries characterized by a low temperature specific expression. When these sequences were used for the BLASTN analysis only 30% and 15% of the entries found a corresponding match in the rice and *Arabidopsis* genomes respectively. These findings could imply that this class of stress-related genes is less conserved than the dehydrin genes between *Triticeae* and other species. Their regulators mechanisms could be different too. The promoter sequence of the low temperature specific barley gene *blt4* contains a CCGAC element identical to that controlling the stress response in *Arabidopsis*, although it was shown to be unable to bind any nuclear protein (Dunn *et al.*, 1998). The fact that the *Arabidopsis* responsive element is inactive in barley suggests that the two species could also have different *trans*-acting factors. These considerations underline the point that, although the molecular response to drought and cold may be generally conserved among all plant species, barley and wheat possess several features that have not been found elsewhere.

Comparative genomics has shown that not only the sequences of stress-related genes are conserved, but the genetic bases of stress tolerance also have a common origin, particularly in closely related genomes such as those of the *Triticeae* and rice. For instance, the map interval on chromosome group 5 containing the *Vrn-A1* locus was shown to be homoeologous to the region of rice chromosome 3 where the heading date QTL *Hd-6* has been mapped (Kato *et al.*, 1999). Deynze *et al.* (1995) also identified a possible relationship between the heading date QTL *Hd3a* of rice and the *Vrn-H2* locus of barley on chromosome 4H. A QTL analysis of chilling tolerance in rice seedlings led to the identification of thirteen QTLs located on rice chromosomes 1, 3, 9 and 11 (Misawa *et al.*, 2000). It is known that rice chromosomes 3, 9 and 11 show large regions of synteny with the homoeologous group 5 chromosome of the *Triticeae*, and three cold tolerance QTLs on rice chromosome 3 are located in the same syntenic interval where *Vrn-1A, Fr1* and *Fr2* in wheat and QTLs for cold tolerance in barley have been mapped. Interestingly, the genomic region controlling the vernalization response and frost tolerance in winter

cereals is orthologous to a region controlling the chilling tolerance in a tropical plant such as rice (Misawa *et al.*, 2000).

Prospects

In the past 10–15 years the molecular dissection of the stress response of barley and wheat has led to the isolation of many genes, several regulatory elements and few transcription factors. Nevertheless, we are still far from fully understanding the molecular basis of stress tolerance/susceptibility. In the near future, thanks to the genomics approach, much more rapid progress towards the identification of signal transduction pathway components is expected.

Genomics, from microarrays to forward and reverse genetics, is recognized as a major revolution in genetic analysis, shifting the focus from the study of a single gene to a whole-genome analysis approach. The rapidly expansion of EST databases makes it possible to collect information on the expressed portion of the genome, opening the way to a better understanding of physiological pathways and to gene discovery, reviewed in Ohlrogge and Benning (2000). This approach has recently been successfully applied to the analysis of genes involved in plant response to stress where modification of gene expression is a common feature (Wood *et al.*, 1999). A number of EST databases have been constructed starting from RNAs of barley or wheat plants exposed to different stress conditions (a list of public available EST databases can be obtained at http://www.tigr.org/tdb/tgi.shtml). ESTs are a starting point for microarray technology that allows the expression of thousands of genes in a single experiment to be monitored providing a global perspective of the response to a stress stimulus (Richmond and Somerville, 2000). The combination of sequence information and RNA expression profiling is a powerful tool for providing correlative evidence on gene functions, although direct demonstration can be only achieved by genetic approaches. Beside the traditional strategy based on the production of transgenic plants over-expressing the gene of interest (gain-of-function approach), new genetic strategies based on the analysis of genotypes in which a given gene is inactivated (loss-of-function approach) are now available. Saturating collections of mutants generated by T-DNA or transposon insertional mutagenesis in *Arabidopsis* and maize (Maes *et al.*, 1999) and the recent development of post-transcriptional gene silencing techniques in tobacco (Baulcombe, 1999) provide new tools for genetic analysis. Mutant analysis represents a crucial step to discover the role of stress-related sequences and their relationship with stress-tolerant loci. As soon as the new genetic tools will become available for barley and wheat, it will be possible to provide evidence on the importance of stress tolerance genes for crop field performance, allowing the design of new breeding strategies for plant improvement.

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References

Abe, H., Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Hosokawa, D. and Shinozaki, K. 1997. Role of *Arabidopsis* MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. Plant Cell 9: 1859–1868.

Anderberg, J.R. and Walker-Simmons, M.K. 1992. Isolation of a wheat cDNA clone for an abscisic acid-inducible transcript with homology to protein kinases. Proc. Natl. Acad. Sci. USA 89: 10183–10187.

Baker, S.S., Wilhelm, K.S. and Thomashow, M.F. 1994. The 5′-region of *Arabidopsis thaliana cor15a* has *cis*-acting elements that confer cold-, drought- and ABA-regulated gene expression. Plant Mol. Biol. 24: 701–713.

Baldi, P., Grossi, M., Pecchioni, N., Valé, G. and Cattivelli, L. 1999. High expression level of a gene coding for a chloroplastic amino acid selective channel protein is correlated to cold acclimation in cereals. Plant Mol. Biol. 41: 233–243.

Bartels, D., Engelhardt, K., Roncarati, R., Schneider, K., Rotter A.M. and Salamini, F. 1991. An ABA and GA modulated gene expressed in the barley embryo encodes an aldose reductase related protein. EMBO J. 10: 1037–1043.

Baulcombe, D.C. 1999. Fast forward genetics based on virus-induced gene silencing. Curr. Opin. Plant Biol. 2: 109–113.

Berberich, T., Uebeler, M. and Feierabend, J. 2000. cDNA cloning of cytoplasmic ribosomal protein S7 of winter rye (*Secale cereale*) and its expression in low-temperature-treated leaves. Biochim. Biophys. Acta 1492: 276–279.

Blum, A. 1989. Osmotic adjustment and growth in barley genotypes under drought stress. Crop Sci. 29: 230–233.

Busk, P.K., Jensen, A.B. and Pagès, M. 1997. Regulatory elements *in vivo* in the promoter of the abscisic acid responsive gene *rab17* from maize. Plant J. 11: 1285–1295.

Cattivelli, L. and Bartels, D. 1990. Molecular cloning and characterization of cold-regulated genes in barley. Plant Physiol. 93: 1504–1510.

Cattivelli, L., Delogu, G., Terzi, V. and Stanca, A.M. 1994. Progress in barley breeding. In: Genetic Improvement of Field Crops. G.A. Slafer (Eds.) Marcel Dekker, New York, pp. 95–181.

Chauvin, L.P., Houde, M. and Sarhan, F. 1993. A leaf-specific gene stimulated by light during wheat acclimation to low temperature. Plant Mol. Biol. 23: 255–265.

Choi, D.W., Koag, M.C. and Close, T.J. 1999. The barley (*Hordeum vulgare* L.) dehydrin multigene family: sequences, allele types, chromosome assignments, and expression characteristics of 11 *Dhn* genes of cv. Dictoo. Theor. Appl. Genet 98: 1234–1247.

Choi, D.W., Koag, M.C. and Close, T.J. 2000. Map locations of barley *Dhn* genes determined by gene-specific PCR. Theor. Appl. Genet. 101: 350–354.

Crosatti, C., Soncini, C., Stanca, A.M. and Cattivelli, L. 1995. The accumulation of a cold-regulated chloroplastic protein is light-dependent. Planta 196: 458–463.

Crosatti, C., Nevo, E., Stanca, A.M. and Cattivelli, L. 1996. Genetic analysis of the COR14 proteins accumulation in wild (*Hordeum spontaneum*) and cultivated (*Hordeum vulgare*) barley. Theor. Appl. Genet. 93: 975–981.

Crosatti, C., Polverino de Laureto, P., Bassi R. and Cattivelli, L. 1999. The interaction between cold and light controls the expression of the cold-regulated barley gene *cor14b* and the accumulation of the corresponding protein. Plant Physiol. 119: 671–680.

Cuming, A.C. 1984. Developmental regulation of gene expression in wheat embryos. Molecular cloning of a DNA sequence encoding the early methionine-labelled (*Em*) polypeptide. Eur. J. Biochem. 145: 351–357.

Curry, J., Morris, C.F. and Walker-Simmons, M.K. 1991. Sequence analysis of a cDNA encoding a group 3 LEA mRNA inducible by ABA or dehydration stress in wheat. Plant Mol. Biol. 16: 1073–1076.

Danyluk J., Perron, A., Houde, M., Limin, A., Fowler, B., Benhamou, N. and Sarhan, F. 1998. Accumulation of an acidic dehydrin in the vicinity of the plasma membrane during cold acclimation of wheat. Plant Cell 10: 623–638.

Dubcovsky, J., Maria, G.S., Epstein, E., Luo, M.C. and Dvorak, J. 1996. Mapping of the K^+/Na^+ discrimination locus in wheat. Theor. Appl. Genet. 92: 448–454.

Dubcovsky J., Lijavetzky, D., Appendino L. and Tranquilli, G. 1998. Comparative mapping of *Triticum monococcum* genes controlling vernalization requirement. Theor. Appl. Genet. 97: 968–975.

Dunn M.A., Hughes, M.A., Pearce, R.S. and Jack, P.L. 1990. Molecular characterization of a barley gene induced by cold treatment. J. Exp. Bot. 41: 1405–1413.

Dunn M.A., Hughes, M.A., Zhang, L., Pearce, R.S., Quigley, AS. and Jack, P.L. 1991. Nucleotide sequence and molecular analysis of the low-temperature induced cereal gene *blt4*. Mol. Gen. Genet. 229: 389–394.

Dunn, M.A., Morris, A., Jack P.L. and Hughes, M.A. 1993. A low-temperature-responsive translation elongation factor 1α from barley (*Hordeum vulgare* L). Plant Mol. Biol. 23: 221–225.

Dunn M.A., Goddard, N.J., Zhang, L., Pearce, R.S. and Hughes, M.A. 1994. Low-temperature-responsive barley genes have different control mechanisms. Plant Mol. Biol. 24: 879–888.

Dunn, M.A., Brown, K., Lightowers, R.L. and Hughes, M.A. 1996. A low-temperature-responsive gene from barley encodes a protein with single stranded nucleic acid binding activity which is phosphorylated in vitro. Plant Mol. Biol. 30: 947–959.

Dunn, M.A., White, A.J., Vural, S and Hughes, M.A. 1998. Identification of promoter elements in a low-temperature-responsive gene (*blt4.9*) from barley (*Hordeum vulgare* L.). Plant Mol. Biol. 38: 551–564.

Dure, L. III 1993. A repeating 11-mer amino acid motif and plant desiccation. Plant J. 3: 363–369.

Ellis, R., Forster, B.P., Waugh, R., Bonar, N., Handley, L.L., Robinson, D., Gordon, D.C. and Powell, G. 1997. Mapping physiological traits in barley. New Phytol. 137: 149–157.

Espelund M., Saeboe-Larssen, S., Hughes, D.W., Galau, G.A., Larsen F. and Jakobsen, K. 1992. Late embryogenesis-abundant genes encoding proteins with different numbers of hydrophilic repeats are regulated differentially by abscisic acid and osmotic stress. Plant J. 2: 241–252.

Faris J.D., Li, W.L., Liu, D.J., Chen, P.D. and Gill, B.S. 1999. Candidate gene analysis of quantitative disease resistance in wheat. Theor. Appl. Genet. 98: 219–225.

Farquhar, G.D. and Richardson, R.A. 1984. Isotopic composition of plants correlates with water use efficiency of wheat genotypes. Aust. J. Plant Physiol. 11: 539–552.

Forster, B.P., Phillips, M.S., Miller, T.E., Baird, E. and Powell, W. 1990. Chromosome location of genes controlling tolerance to salt (NaCl) and vigour in *Hordeum vulgare* and *H. chilense*. Heredity 65: 99–107.

Fowler D.B., Chauvin, L.P., Limin, A.E. and Sarhan, F. 1996. The regulatory role of vernalization in the expression of low-temperature-induced genes in wheat and rye. Theor. Appl. Genet. 93: 554–559.

Franckowiak, J. 1997. Revised linkage maps for morphological markers in barley, *Hordeum vulgare*. Barley Genet. Newsl. 26: 9–21.

Fridman, E., Pleban, T. and Zamir, D. 2000. A recombinant hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene Proc. Natl. Acad. Sci. USA 97: 4718–4723.

Gai, X.W., Lal, S., Xing, L.Q., Brendel, V. and Walbot, V.2000. Gene discovery using the maize genome database ZmDB. Nucl. Acids Res. 28: 94–96.

Galiba G., Quarrie, S.A., Sutka, J., Morgounov, A. and Snape, J.W. 1995. RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. Theor. Appl. Genet. 90: 1174–1179.

Gallagher, L.W., Solimann, K.M., Vivar, H. 1991. Interactions among loci conferring photoperiod insensitivity for heading time in spring barley. Crop Sci. 31: 256–261.

Gana, J.A., Sutton, F. and Kenefick, D.G. 1997. cDNA structure and expression patterns a low-temperature-specific wheat gene *tacr7*. Plant Mol. Biol. 34: 643–650.

Gilmour, S.J., Zarka, D.G., Stockinger, E.J., Salazar, M.P., Houghton, J.M. and Thomashow, M.F. 1998. Low temperature regulation of the *Arabidopsis* CBF family of AP2 transcriptional activators as an early step in cold induced COR gene expression. Plant J. 16: 433–443.

Goddard, N.J., Dunn, M.A., Zhang, L., White, A.J., Jack, P.L. and Hughes, M.A. 1993. Molecular analysis and spatial expression pattern of a low-temperature-specific barley gene, *blt101*. Plant Mol. Biol. 23: 871–879.

Gorny, A.G. 1999. Effects of D-genome substitutions on the water use efficiency and of the 'Langdon' durum wheat to reduced nitrogen nutrition. Cereal Res. Comm. 27: 83–90.

Grossi, M., Gulli, M., Stanca, A.M. and Cattivelli, L. 1995. Characterization of two barley genes that respond rapidly to dehydration stress. Plant Sci. 105: 71–80.

Grossi, M., Giorni, E., Rizza, F., Stanca, A.M. and Cattivelli, L. 1998. Wild and cultivated barleys show differences in the expression pattern of a cold-regulated gene family under different light and temperature conditions. Plant Mol. Biol. 38: 1061–1069.

Guiltinan, M.J., Marcotte, W.R. Jr, and Quatrano, R.S. 1990. A plant leucine zipper protein that recognizes an abscisic acid-responsive element. Science 250: 267–271.

Handley, L.L., Nevo, E., Raven, J.A., Martines-Carrasco, R., Scrimgeour, C.M., Pakniyat, H. and Forster, B.P. 1994. Chromosome

4 controls potential water use efficiency (δ^{13}) in barley. J. Exp. Bot. 45: 1661-663.

Hayes P.M., Blake, T., Chen, T.H.H., Tragoonrung, S., Chen, F., Pan, A. and Liu, B. 1993. Quantitative trait loci on barley (*Hordeum vulgare* L.) chromosome-7 associated with components of winterhardiness. Genome 36: 66–71.

Hockett, E.A. and Nilan, R.A. 1985. Genetics. In: D.C. Rasmusson (Ed.), Barley, ASA/CSSA/SSSA, Madison, WI, pp. 187–230.

Hollung, K., Espelund, M. and Jakobsen, K.S. 1994. Another Lea B19 gene (Group 1 barley containing a single 20 amino acid hydrophilic motif Plant Mol. Biol. 25: 559–564.

Hong, B., Barg R. and Ho, T.-O.. 1992. Developmental and organ-specific expression of an ABA- and stress-induced protein in barley. Plant Mol. Biol. 18: 663–674.

Houde, M., Danyluk, J., Laliberté, J-F., Rassart, E., Dhindsa, R.S. and Sarhan, F. 1992. Cloning, characterization, and expression of a cDNA encoding a 50-Kilodalton protein specifically induced by cold acclimation in wheat. Plant Physiol. 99: 1381–1387.

Ishitani, M., Nakamura, T., Han, S.Y. and Takabe, T. 1995. Expression of the betaine aldehyde dehydrogenase gene in barley in response to osmotic stress and abscisic acid. Plant Mol. Biol. 27: 307–315.

Ismail, A.M., Hall, A.E. and Close, T.J. 1999. Allelic variation of a dehydrin gene cosegregates with chilling tolerance during seedling emergence. Proc. Natl. Acad. Sci. USA 96: 13566–13570.

Jiang, C., Iu, B., Singh, J. 1996. Requirement of a CCGAC *cis*-acting element for cold induction of BN115 gene from *B. napus*. Plant Mol. Biol. 30: 679–684.

Joshee, N., Kisaka, H. and Kitagawa, Y. 1998. Isolation and characterization of a water stress-specific genomic gene, *pwsi18*, from rice. Plant Cell Physiol. 39: 64–72.

Joshi, C.P., King, S.W., Nguyen, H.T. 1992. Molecular cloning and characterization of a cDNA encoding a water stress protein (*wsp23*) from wheat roots. Plant Sci. 86: 71–82.

Kasai K., Fukayama, H., Uchida, N., Mori, N., Yasuda, T., Oji, Y. and Nakamura, C. 1998. Salinity tolerance in *Triticum aestivum-Lophopyrum elongatum* amphiploid and 5E disomic addition line evaluated by NaCl effects on photosynthesis and respiration. Cereal Res. Comm. 26: 281–287.

Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nature Biotechnol. 17: 287–291.

Kato K., Miura H. and Sawada, S. 1999. Comparative mapping of the wheat Vrn-A1 region with the rice *Hd-6* region. Genome 42: 204–209.

King, S.W., Joshi, C.P. and Nguyen, H.T. 1992. DNA sequence of an ABA responsive gene (*rab15*) from water-stressed wheat roots. Plant Mol. Biol. 18: 119-121.

Knight, CD., Sehgal, A., Atwal, K., Wallace, J.C., Cove, D.J., Coates, D., Quatrano, R.S., Bahadur, S., Stockley, P.G. and Cuming, A. 1995. Molecular responses to abscisic acid and stress are conserved between moss and cereals. Plant Cell 7: 499–506.

Knight, H., Veale, E.L., Warren, G.J. and Knight, M.R. 1999. The *sfr6* mutation in *Arabidopsis* suppresses low-temperature induction of genes dependent on the CRT DRE sequence motif. Plant Cell 11: 875–886.

Koebner, R.M.D., Martin, P.K., Orford, S.M. and Miller, T.E. 1996. Responses to salt stress controlled by the homeologous group 5 chromosomes of hexaploid wheat. Plant Breed. 115: 81–84.

Koike, M., Takezawa, D., Arakawa, K. and Yoshida, S. 1997. Accumulation of 19-kDa plasma membrane polypeptide during induction of freezing tolerance in wheat suspension-cultured cells by abscisic acid. Plant Cell Physiol. 38: 707–716.

Kurek, I., Harvey, A.J., Lonsdale, D.M. and Breiman, A. 2000. Isolation and characterization of the wheat prolyl isomerase FK506-binding protein (FKBP) 73 promoter. Plant Mol. Biol. 42: 489–497.

Labhilili, M., Jourdier, P. and Gautier, M-F .1995. Characterization of cDNAs encoding *Triticum durum* dehydrins and their expression patterns in cultivars that differ in drought tolerance. Plant Sci. 112: 219–230.

Langridge, P., Karakousis, A., Collins, N., Kretschmer J. and Manning, S. 1995. A consensus linkage map of barley (*Hordeum vulgare* L.). Mol. Breed. 1: 389–395.

Laurie, D.A. 1997. Comparative genetics of flowering time. Plant Mol. Biol. 35: 167–177.

Laurie, D.A., Pratchett, N., Bezant, J.H. and Snape, J.W. 1994. Genetic analysis of a photoperiod response gene on the short arm of chromosome 2(2H) of *Hordeum vulgare* (barley). Heredity 72: 619–627.

Laurie, D.A., Pratchett, N., Bezant, J.H. and Snape, J.W. 1995. RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38: 575–585.

Law, C.N. 1966. The location of genetic factors affecting a quantitative character in wheat. Genetics 53: 487–493.

Leung, J. and Giraudat, J. 1998. Abscisic acid signal transduction. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49: 199–222.

Li, Z.Y. and Chen, S.Y. 2000. Isolation and characterization of a salt- and drought-inducible gene for *S*-adenosylmethionine decarboxylase from wheat (*Triticum aestivum* L.). Plant Physiol. 156: 386–393.

Lilley, J.M., Ludlow, M.M., McCouch, S.R. and O'Toole, J.C. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. J. Exp. Bot. 47: 1427–1436.

Litts, J.C., Colwell, G.W., Chakerian, R.L. and Quatrano, R.S. 1987. The nucleotide sequence of a cDNA clone encoding the wheat *Em* protein. Nucl. Acids. Res. 15: 3607–3618.

Liu, Q., Sakuma, Y., Abe, H., Kasuga, M., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. 1998. Two transcriptional factors, DREB1 and DREB2, with an EREP/AP2 DNA binding domain, separate two cellular signal transduction pathways in drought- and low temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell 10: 1391–1406.

Maes, T., De Keukeleire, P. and Gerats, T. 1999. Plant tagnology. Trends Plant Sci. 4: 90–96.

Mano, Y. and Takeda, K. 1997. Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). Euphytica 94: 263–272.

Marcotte, W.R. Jr, Russel, S.H. and Quatrano, R.S. 1989. Abscisic acid response sequences from the *Em* gene of wheat. Plant Cell 1: 969–976.

Mastrangelo, A.M., Baldi, P., Maré, C., Terzi, V., Galiba, G., Cattivelli, L. and Di Fonzo, N. 2000. The cold dependent accumulation of COR TMC-AP3 in cereals with contrasting frost tolerance is regulated by different mRNA expression and protein turnover. Plant Sci. 156: 47–54.

McIntosh, R.A., Hart, G.E. and Gale, M.D. 1998. Catalogue of gene symbols for wheat. Proceedings 9th International Wheat Genetics Symposium, Saskatchewan, Canada, pp. 1333–1500.

Mekhedov, S., de Ilarduya, O.M. and Ohlrogge, J. 2000. Toward a functional catalogue of the plant genome. A survey of genes for lipid biosynthesis. Plant Physiol. 122: 389–401.

Misawa, S., Mori, N., Takumi, S., Yoshida, S. and Nakamura, C. 2000. Mapping of QTLs for low temperature response in seedling of rice (*Otyza sativa* L.). Cereal Res. Comm. 28: 33–41.

Molina-Cano, J.L., Sopena, A., Swanston, J.S., Casas, A.M., Moralejo, M.A., Ubieto, A., Lara, I., Perez-Vendrell, A.M. and Romagosa, 1.1999. A mutant induced in the malting barley cv. Triumph with reduced dormancy and ABA response. Theor. Appl. Genet. 98: 347–355.

Morgan, J.M. and Tan, M.K. 1996. Chromosomal location of a wheat osmoregulation gene using RFLP analysis. Aust. J. Plant Physiol. 23: 803–806.

Mudgett, M.B. and Clarke, S. 1994. Hormonal and environmental responsiveness of a developmentally regulated protein repair L-isoaspartyl methyltransferase in wheat. J. Biol. Chem. 269: 25605–25612.

Mullarkey, M. and Jones, P. 2000. Isolation and analysis of thermotolerant mutants of wheat. J. Exp. Bot. 51: 139–146.

Mundy, J., Yamaguchi-Shinozaki, K. and Chua, N-H. 1990. Nuclear proteins bind conserved elements in the abscisic acid-responsive promoter of a rice rab gene. Proc. Natl. Acad. Sci. USA 87: 1406–1410.

Muramoto, Y., Watanabe, A., Nakamura, T. and Takabe, T. 1999. Enhanced expression of a nuclease gene in leaves of barley plants under salt stress. Gene 234: 315–321.

Nakagawa, H., Ohmiya, K. and Hattori, T. 1996. A rice bZIP protein, designated OSBZ8, is rapidly induced by abscisic acid. Plant J. 9: 217–227.

Nemoto, Y., Kawakami, N. and Sasakuma, T. 1999. Isolation of novel early salt-responsive genes from wheat (*Triticum aestivum* L.) by differential display. Theor. Appl. Genet. 98: 673–678.

Nuccio, M..L., Rhodes, D., McNeil, S.D. and Hanson, A.D. 1999. Metabolic engineering of plants for osmotic stress resistance. Curr. Opin. Plant Biol. 2: 128–134.

Ohlrogge, J. and Benning, C. 2000. Unravelling plant metabolism by EST analysis. Curr. Opin. Plant Biol. 3: 224–228.

Ouellet, F., Vazquez-Tello, A. and Sarhan, F. 1998. The wheat *wcs120* promoter is cold-inducible in both monocotyledonous and dicotyledonous species. FEBS Lett. 423: 324–328.

Pan, A., Hayes, P.M., Chen, F., Chen, T.H.H., Blake, T., Wright, S., Karsai, I. and Bedö, Z. 1994. Genetic analysis of the components of winterhardiness in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 89: 900–910.

Pearce, R.S., Houlston, C.E., Atherton, K.M., Rixon, J.E., Harrison, P., Hughes, M.A. and Dunn, M.A. 1998. Localization of expression of three cold-induced genes, *blt101, blt4.9* and *blt14*, in different tissues of the crown and developing leaves of cold-acclimated cultivated barley. Plant Physiol. 117: 787–795.

Phillips, J.R., Dunn, M.A. and Hughes, M.A. 1997. mRNA stability and localisation of the low-temperature-responsive gene family *blt14*. Plant Mol. Biol. 33: 1013–1023.

Plaschke, J., Borner, A., Xie, D.X., Koebner, R.D.M., Schlegel, R. and Gale, M.D. 1993. RFLP mapping of genes affecting plant height and growth habit in rye. Theor. Appl. Genet. 85: 1049–1054.

Pugsley, A.T. 1973. Control of developmental patterns in wheat through breeding. Proc. 4th International Wheat Genetics Symposium, pp. 857–859.

Qi, X., Stam, P. and Lindhout, P. 1996. Comparison and integration of four barley genetic maps. Genome 39: 379–394.

Quarrie, S.A., Gulli, M., Calestani, C., Steed, A. and Marmiroli, N. 1994. Location of a gene regulation drought-induced abscisic acid production on the long arm of chromosome 5A of wheat. Theor. Appl. Genet. 89: 794–800.

Rascio, A., Russo, M., Mazzucco, L., Platani, C., Nicastro, G. and Di Fonzo, N. 2001. Enhanced osmotolerance of a wheat mutant selected for potassium accumulation. Plant Sci. 160: 441–448.

Rascio, A., Russo, M., Platani, C., Ronga, G. and Di Fonzo, N. 1999. Mutants of durum wheat with alterations in tissue affinity for strongly bound water. Plant Sci. 144: 29–34.

Raskin, I., and Ladyman, J.A.R. 1988. Isolation and characterization of a barley mutant with abscisic-acid-insensitive stomata. Planta 173: 73-78.

Richmond, T. and Somerville, S. 2000. Chasing the dream: plant EST microarray. Curr. Opin. Plant Biol. 3: 108–116.

Saijo, Y., Hata, S., Kyozuka, J., Shimamoto, K. and Izui, K. 2000. Over-expression of a single Ca^{2+}-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. Plant J. 23: 319–327.

Sarhan, F. and Danyluk, J. 1998. Engineering cold-tolerant crops throwing the master switch. Trends Plant Sci. 3: 289–290.

Shen, Q. and Ho, T.-H.D. 1995. Functional dissection of an abscisic acid (ABA)-inducible gene reveals two independent ABA-responsive complexes each containing a G-box and a novel *cis*-acting element. Plant Cell 7: 295–307.

Shen, Q., Zhang, P.N. and Ho, T.-H.D. 1996. Modular nature of abscisic acid (ABA) response complexes: composite promoter units that are necessary and sufficient for ABA induction of gene expression in barley. Plant Cell 8: 1107–1119.

Shimosaka, E., Sasauma, T. and Handa, H. 1999. A wheat cold-regulated cDNA encoding an early light-inducible protein (ELIP): its structure, expression and chromosomal location. Plant Cell Physiol. 40: 319–325.

Sivamani, E., Bahieldin, A., Wraith, J.M., Al-Niemi, T., Dyer, W.E., Ho, T.D. and Qu, R. 2000. Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley *HVA1* gene. Plant Sci. 155: 1–9.

Skriver, K., Olsen, P.L., Rogers, J.C. and Mundy, J. 1991. *Cis*-acting DNA elements responsive to gibberellin and its antagonist abscisic acid. Proc. Natl. Acad. Sci. USA 88: 7266–7270.

Snape, J.W., Quarrie, S.A., and Laurie, D.A. 1996. Comparative mapping and its use for the genetic analysis of agronomic characters in wheat. Euphytica 89: 27–31.

Snape, J.W., Semikhodskii, A., Fish, L., Sarma, R.N., Quarrie, S.A., Galiba, G. and Sutka J. 1997. Mapping frost tolerance loci in wheat and comparative mapping with other cereals. Acta Agric. Hung. 45: 265–270.

Stokinger, E.J., Gilmour, S.J., and Thomashow, M.F. 1997. *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a *cis*-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc. Natl. Acad. Sci. USA 94: 1035–1040.

Storlie, E.W., Allan, R.E. and Walker-Simmons, M.K. 1998. Effect of the *Vrn1-Fr1* interval on cold hardiness levels in near-isogenic wheat lines. Crop Sci. 38: 483–488.

Straub, P.F., Shen, Q., Ho, T.-H.D. 1994. Structure and promoter analysis of an ABA- and stress-regulated barley gene HVA1. Plant Mol. Biol. 26: 617–630.

Sutka, J., Galiba, G., Vaguifalvi, A., Gill, B.S. and Snape, J.W. 1999. Physical mapping of the Vrn-A1 and Fr1 genes on chromosome 5A of wheat using delition lines. Theor. Appl. Genet. 99: 199–202.

Sutka, J. and Snape, J.W. 1989. Location of a gene for frost resistance on chromosome 5A of wheat. Euphytica 42: 41–44.

Sutton, F., Ding, X. and Kenefrik, D.G. 1992. Group 3 Lea genes HVA1 regulation by cold acclimation and deacclimation in two

barley cultivars with varying freeze resistance. Plant Physiol. 99: 338–340.

Swire-Clark, G.A. and Marcotte, W.R. 1999. The wheat LEA protein Em functions as an osmoprotective molecule in *Saccharomyces cerevisiae*. Plant Mol. Biol. 39: 117–128.

Teulat, B., Monneveux, P., Wery, J., Borries, C., Souyris, I., Charrier, A. and This D. 1997. Relationship between relative water content and growth parameters under water stress in barley: a QTL study. New Phytol. 137: 99–107.

Teulat, B., This, D., Khairallah, M., Borries, C., Ragot, C., Sourdille, P., Leroy, P., Monneveux P. and Charrier, A. 1998. Several QTLs involved in osmotic-adjustment trait variation in barley. Theor. Appl. Genet. 96: 688–698.

Tuberosa, R., Galiba, G., Sanguineti, M.C., Noli E. and Sutka, J. 1997. Identification of QTL influencing freezing tolerance in barley. Acta Agric. Hung. 45: 413–417.

Vagujfalvi, A., Crosatti, C., Galiba, G., Dubcovsky J. and Cattivelli L. 2000. Two loci on wheat chromosome 5A regulate the differential cold-dependent expression of the *cor14b* gene in frost-tolerant and frost-sensitive genotypes. Mol. Gen. Genet. 263: 194–200.

Van Deynze, A.E., Nelson, J.C., Yglesias, E.S., Harrington, S.E., Braga, D.P., McCouch, S.R. and Sorrells, M.E. 1995. Comparative mapping in grasses: wheat relationships. Mol. Gen. Genet. 248: 748–754.

Wood, A.J., Duff, R.J. and Oliver, M.J. 1999. Expressed sequence tags (ESTs) from desiccated *Tortula ruralis* identify a large number of novel plant genes. Plant Cell Physiol. 40: 361–368.

Worland, A.J. 1996. The influence of flowering time genes on environmental adaptability in European wheats. Euphytica 89: 49–57.

Xin, Z. and Browse, J. 1998. *eskimo1* mutants of *Arabidopsis* are constitutively freezing-tolerant. Proc. Natl. Acad. Sci. USA 95: 7799–7804.

Xu, K. and Mackill, D.J. 1996. A major locus for submergence tolerance mapped on rice chromosome 9. Mol. Breed. 2: 219–224.

Xu, D., Duan, X., Wang, B., Hong, B., Ho, T.-H.D. and Wu, R. 1996. Expression of late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol. 110: 249–257.

Yamaguchi-Shinozaki, K. and Shinozaki, K. 1994. A novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low temperature or high-salt stress. Plant Cell 6: 251–264.

Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Yamamoto K., Umehara Y., Nagamura Y. and Sasaki T. 2000. *Hd1*, major photoperiod-sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANT*. Plant Cell 12: 2473–2484.

Yet, S., Moffatt, B.A., Griffith, M., Xiong, F., Yang, D.S.C., Wiseman, S.B., Sarhan, F., Danyluk, J., Xue, Y.Q., Hew, C.L., Doeherty-Kirby, A. and Lajoie G. 2000. Chitinase genes responsive to cold encode antifreeze proteins in winter cereals. Plant Physiol. 124: 1251–1263.

Zhu, B., Choi, D-W., Fenton, R. and Close, T.J. 2000. Expression of the barley dehydrin multigene family and the development of freezing tolerance. Mol. Gen. Genet. 264: 145–153.