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Chromosome mapping of low-temperature induced *Wcs120* family genes and regulation of cold-tolerance expression in wheat

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Abstract Low-temperature (LT) induced genes of the *Wcs120* family in wheat (*Triticum aestivum*) were mapped to specific chromosome arms using Western and Southern blot analysis on the ditelocentric series in the cultivar Chinese Spring (CS). Identified genes were located on the long arms of the homoeologous group 6 chromosomes of all 3 genomes (A, B, and D) of hexaploid wheat. Related species carrying either the A, D, or AB genomes were also examined using Southern and Western analysis with the *Wcs120* probe and the WCS120 antibody. All closely related species carrying one or more of the genomes of hexaploid wheat produced a 50 kDa protein that was identified by the antibody, and a *Wcs120* homoeologue was detected by Southern analysis in all species. In the absence of chromosome arm 6DL in hexaploid CS wheat no 50 kDa protein was produced and the high-intensity *Wcs120* band was missing, indicating 6DL as the location of *Wcs120* but suggesting silencing of the *Wcs120* homoeologue in the A genome. Levels of proteins that cross-reacted with the *Wcs120* antibody and degrees of cold tolerance were also investigated in the Chinese Spring/Cheyenne (CS/CNN) chromosome substitution series. CNN chromosome 5A increased the cold tolerance of CS wheat. Densitometry scanning of Western blots to determine protein levels showed that the group 5 chromosome 5A had a regulatory effect on the expression of the *Wcs120* gene family located on the group 6 chromosomes of all three hexaploid wheat genomes.

Key words Gene regulation · Cold tolerance · Triticeae · Gene mapping · Gene silencing

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Introduction

Plants with the ability to develop freezing tolerance are able to acclimate to cold during exposure to low, but above freezing temperatures. Cold acclimation is cumulative with time and involves the synthesis, or increased accumulation, of particular subsets of proteins in plants (Guy 1990, Houde et al. 1992a). Some of these low-temperature (LT) induced proteins share common characteristics (Welin et al. 1944) with other 'stress-related' plant proteins that are induced by drought, salt stress, abscisic acid (ABA), or during seed development (dehydrins, Close and Chandler 1990; Close et al. 1995; early salt stress induced, Gulick and An 1993; ABA responsive and late embryogenesis abundant, Dure et al. 1989). Shared characteristics are variable but involve highly conserved repeat amino acid sequences, one of which is rich in glycine and is peculiar to the N-terminal portion while the other is a basic lysine-rich repeat peculiar to the C-terminal portion of the protein. These proteins are hydrophilic and remain soluble upon boiling. No definitive function has been assigned to this group of proteins but all of the induction stimuli ultimately prepare the plant for some form of dehydration stress. Cold-acclimating plants reduce their water content and, upon exposure to freezing temperatures, move water from the cell protoplasm to intercellular ice crystals, causing severe dehydration stress within the cells (Levitt 1980; Pearce 1988).

These stress-related proteins may be induced by one or more stimuli that may act via different pathways (Welin et al. 1994). Thus, a LT-induced wheat gene *Wcs120* (Houde et al. 1992a, b) was found to be constitutively expressed at very low levels and to have only low levels of transcript accumulation in response to ABA or drought. Anti-WCS120-antibodies directed against the protein product cross-react with a group of

low-temperature induced wheat proteins that appear to be coordinately regulated and correlated with the development and degree of freezing tolerance.

The chromosomal location of several dehydrin genes has been established in the diploid barley species *Hordeum vulgare* (Close and Chandler 1990; Pan et al. 1994). Common wheat (*Triticum aestivum*) and *H. vulgare* are closely related, belonging to the same taxonomic group (Triticeae). *T. aestivum* is a hexaploid comprised of three genomes designated A, B, and D. Two of the diploid species contributing these genomes are *Triticum monococcum* (A genome; evidence reviewed by Kerby et al. 1988) and *Triticum tauschii* (D genome) (McFadden and Sears 1946). The origin of the B genome is uncertain (Kerby and Kuspira 1988) and could be multispecific due to interspecific genome hybridization involving members of a similar taxonomic group (Kimber and Athwal 1972).

In this paper we localize genes and gene products of the *Wcs120* family to the ancestral diploids; map this family of genes to modern hexaploid wheat (*T. aestivum*) using the ditelocentric (DT) series developed in 'Chinese Spring' (CS) wheat, and study expression of the protein products in a chromosomal substitution series involving nonhardy CS and the more cold-tolerant wheat cultivar 'Cheyenne' (CNN).

Materials and methods

Plant genetic stocks

Species identifications, cultivar or accession number, abbreviations, and genomic formulae are listed in Table 1. In the ditelocentric (DT) series of *T. aestivum* cv. Chinese Spring (CS), CSDT, all chromosomes are present but in each line one chromosome pair is represented by only the telocentric chromosomes of one arm (provided by the USDA from E. R. Sears collection). The presence of the long or short arm of the chromosome is indicated by 'L' or 'S', respectively. CSDT lines 2AL, 2BS, 4AS, and the group 5 'S' lines were unavailable at the time of this experiment. The Chinese Spring/ Cheyenne (CS/CNN) wheat chromosome substitution series of 21 lines, in which an individual pair of homologous chromosomes of CS is replaced by the corresponding homologous chromosome pair of the more cold-tolerant winter wheat cultivar Cheyenne (CNN), was kindly provided by Rosalind Morris, University of Nebraska.

Growth and LT acclimation conditions

Plant materials for Southern analysis were grown in pots at 20° C on a 16 h photoperiod for 42 days. Seed of material intended for LT acclimation was germinated at 24° C for 3 days then placed in hydroponic trays containing half-strength Hoagland's nutrient solution. Growth conditions, thereafter were a constant 17° C with a 16 h photoperiod for 14 days. The LT acclimation period was 3 weeks at 2° C with a 16 h photoperiod for the ditelocentric series in Chinese Spring wheat and 4° C for 3 weeks for all other material. Experimental design for the CS/CNN substitution series was a Randomized Complete Block Design with four replicates.

Southern blot analysis

Genomic DNA was isolated from wheat leaves as previously described (Rogers and Bendich 1988) and DNA concentration was determined using the diphenylamine colorimetric assay (Burton 1968). DNA samples were digested with the restriction endonucleases *Dra*I, *Eco*RV, and *Xba*I, then separated by a FIGE mapper (Field Inversion Gel Electrophoresis, BIO-RAD) on a 1.1% agarose gel. The DNA concentration was adjusted according to the genome size: 0.6, 1.2 and 1.8 μg were used for the diploid, tetraploid and hexaploid wheats, respectively. After the electrophoresis, the gel was soaked for 1 h in a denaturing solution (0.5 M NaOH, 1.5 M NaCl) and neutralized for 1 h in 1 M TRIS-HCl pH 7.5, 1.5 M NaCl. DNA was then transferred to a nitrocellulose membrane and hybridized with the ^{32}P-labelled *Wcs120* probe. After hybridization, blots were washed under high stringency conditions (0.1 x SSC at 69° C) and exposed to X-Omat- AR film (Eastman-Kodak).

Protein extraction and immunoblot analysis

Soluble proteins were extracted from leaf tissue by grinding in a precooled mortar in buffer containing 0.1 M TRIS-HCl pH 9.6, 1 mM PMSF (3 ml buffer/g of tissue). The extract was immediately centrifuged for 5 min at 12000 × *g* and the supernatant was removed and solubilized in an equal volume of 2 x electrophoresis sample buffer. Protein concentration was estimated using the Bio-Rad *DC* Protein Assay. Proteins (5 μg/lane) were separated on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane (Hybond-C *Extra*Amersham). The membranes were blocked in a

Table 1 Plant genomic stocks used, and sizes (kDa) of proteins identified by the WCS120 antibody

Species	Genome	Cultivar/accession	Abbreviation	200	180	66	50
Triticum aestivum L. em. Thell	ABD	Norstar	NOR	X	X	X	X
		Chinese Spring	CS	X	X	X	X
		Cheyenne	CNN	X	X	X	X
Triticum turgidum L. var. durum	AB	Michurinka	MICH		X	X	X
		Novamichurinka	NOVA		X	X	X
Triticum dicoccum Schrank	AB	PI355503	TD63	X	X		X
		PI352369	TD44	X		X	X
		PI352367	TD43	X	X		X
Triricum monococcum L.	A	PI330550	TM65	X			X
		PI352475	TM10	X	X		X
Triticum boeoticum Boiss.	A	PI355453	TB3	X	X		X
Triticum tauschii (*Coss.*) Schmal.	D	CI4	SQ4		X		X
(*Aegilops squarrosa* L)		PGR1548	SQ44		X		X
		PGR1549	SQ45		X		X
		RL5257	SQ57		X		X

X indicates presence of the protein

4% (w/v) solution of reconstituted milk powder prepared in PBS containing 0.2% (v/v) polyoxyethylene sorbitan monolaurate (Tween-20). The membranes were probed with an antibody raised against the 50 kDa cold-induced protein from winter wheat (WCS120) at a 1:10000 dilution for 1 h. After washing with PBS-Tween, the proteins recognized by the primary antibody were revealed with horseradish peroxidase coupled to anti-rabbit IgG (Jackson Immunoresearch) as a secondary antibody at 1:25000 dilution. The complex was visualized using the ECL chemiluminescent detection system (Amersham) and X-Omat-RP film (Eastman-Kodak). Relative levels of WCS120 proteins were determined by densitometry (Molecular Dynamics) scanning of the blot as previously described (Houde et al. 1995).

Results

Major proteins cross-reacting with the WCS120 antibody

The LT-responsive *Wcs120* gene family of hexaploid wheat encodes a group of proteins ranging in size from 40 to 200 kDa. These proteins cross-react in hexaploid wheat (Fig. 1, NOR and CS) with an antibody raised against the 50 kDa protein encoded by the *Wcs120* gene. Levels of all proteins recognized were much higher in the more cold-tolerant lines, such as Norstar (NOR), than in less tolerant lines like Chinese Spring (CS) (Fig. 1). This group of proteins has been suggested for use as freezing tolerance markers (Houde et al. 1992a) because their accumulation is associated with cold tolerance.

The ditelocentric series from Chinese Spring Wheat (CSDT), in which one homologous pair of chromosome arms is missing in each line, was used to determine which chromosome arm carried the gene producing any particular protein of this gene family. Western analysis of these lines (Fig. 1, Table 2) revealed that: the 50 kDa protein was missing in DT6DS (Chromosome arm 6DL absent); the 66 kDa protein was missing in DT6BS (chromosome arm 6BL absent); and the 200 kDa protein was missing in DT6AS (chromosome arm 6AL absent). All CSDT lines produced a protein of approximately 180 kDa but careful examination of western blots (Fig. 1) suggests that this band may actually be a doublet with the smaller component of the doublet being absent in 6DS and the larger absent in 6AS. Low accumulation of the 40 kDa protein in the CS lines made the detection of its absence uncertain.

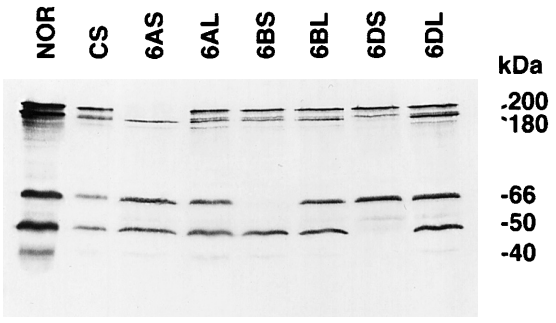

Fig. 1 Immunoblot analysis of soluble proteins isolated from LT-acclimated *T. aestivum* cultivars Norstar (NOR), Chinese Spring (CS), and ditelocentric lines of CS, probed with anti-WCS120 antibody

Table 2 Chromosomal location of *Wcs120* family genes or genes producing proteins recognized by the anti-WCS120 antibody

Chromosome[a]	Gene	Cross-reacting protein products
6DL	*Wcs120*	50 kDa 180 kDa
6BL	*Wcs66*	66 kD
6AL	*Wcs200*	200 kDa 180 kDa

[a]L denotes the long arm of the chromosome

Western analysis with the WCS120 antibody was also performed on progenitor or related species sharing common genomes with wheat (Fig. 2). Prominent proteins (Fig. 2, Table 1) were of 50 and 200 kDa size in the A genome species (TB3, TM65, and TM10), and 50 and 180 kDa in the D genome species (SQ4, SQ45, SQ57, and SQ44). One major distinction was found within the tetraploid AB genome lines: NOVA, MICH, and TD44 accumulated a 66 kDa protein, while TD63 and TD43 showed no indication of this protein. All members of this tetraploid group accumulated a 50 kDa protein and a 180 kDa protein, although the 180 kDa protein was very weak in NOVA and MICH and is not apparent in Fig. 2. The TD accessions also produced a 200 kDa protein that could not be detected in NOVA and MICH.

Southern analysis using the *Wcs120* probe

Southern analysis (Fig. 3) using the endonucleases *Dra*I, *Eco*RV, and *Xba*I detected a high-intensity band in all CS lines except the 6DS line. The lack of this hybridization band, together with the absence of the 50 kDa protein in this same 6DS line, localize *Wcs120* to the long arm of chromosome 6D. *Wcs120* and *Wcs66* are highly homologous genes (Chauvin et al. 1994) and both hybridize with equal intensity to the *Wcs120* probe

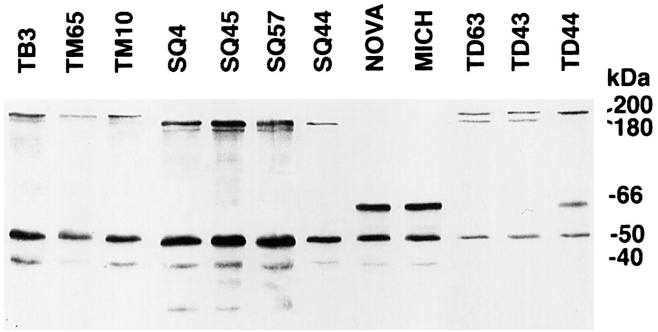

Fig. 2 Immunoblot analysis of soluble proteins isolated from LT-acclimated relatives of *T. aestivum* (ABD genome) probed with anti-WCS120 antibody: A genome (TB3, TM65, and TM10), D genome (SQ4, 45, 57, and 44), AB genome (NOVA, MICH, TD63, TD43, and TD44)

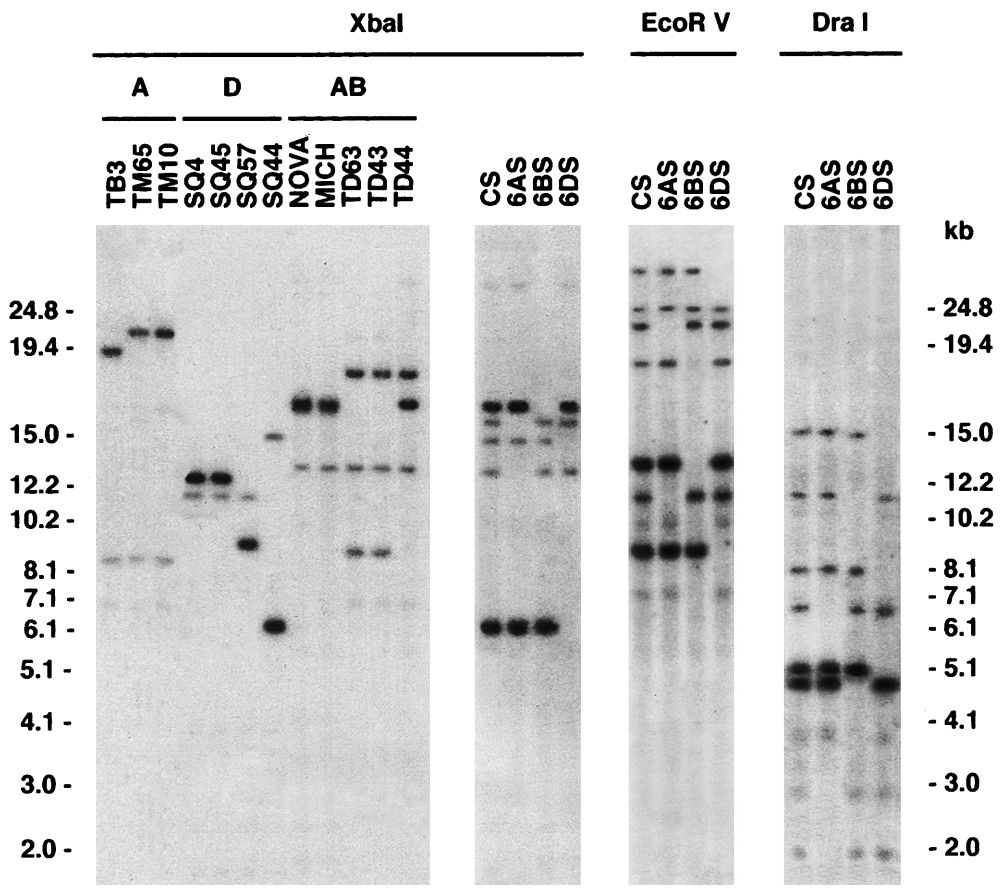

Fig. 3 Southern blot analysis using the *Wcs120* probe on genomic DNA from *T. aestivum* cultivar CS and ditelocentric lines of CS digested with *Xba*I, *Eco*RV and *Dra*I, and (left panel) A, D, and AB genome DNAs digested with *Xba*I

in Southern blots. The high intensity band missing in the 6BS lane of all three digests and the absence of the 66 kDa protein in the 6BS lane of Fig. 1 localize *Wcs66* to the long arm of chromosome 6B. The other members of this gene family show lower homology to the *Wcs120* probe (Ouellet et al. 1993; Houde et al. 1992b) and could not be distinguished from other weakly hybridizing fragments in these digests. Although DT lines 6AS, 6BS, and 6DS were clearly missing less strongly hybridizing bands in Southern blots, it was not possible to definitively identify these genes.

Endonuclease *Xba*I (Fig. 3) was used to compare accessions possessing the A, D, and AB genomes to the hexaploid ABD genome of CS. The D genome accessions each produced one strongly hybridizing band indicating high homology to *Wcs120*. SQ44 produced a band of equal size, as well as intensity, to that found in the hexaploid wheat CS. The tetraploid (AB genome) wheats TD44, MICH, and NOVA each produced a hybridizing band of similar size and intensity to the *Wcs66* band found in the B genome of CS. Tetraploids TD63 and 43 lacked this hybridizing band with *Wcs66* homology. Since this hybridizing band is the *Wcs66* homoeologue, the *Wcs120* homoeologue appears to be the larger hybridizing fragment in TD63, 43, and 44, and part of the doublet at the *Wcs66* location in NOVA and MICH. The larger bands of the A genome accessions with the strongest hybridization intensity appear to be homoeologues of *Wcs120*.

Cold tolerance and WCS120 protein accumulation in chromosome substitution lines

A chromosome substitution series with individual chromosomes of the more cold-tolerant cultivar Cheyenne (CNN) substituted in individual pairs for the corresponding homologous chromosome pair of the nonhardy Chinese Spring (CS) cultivar, along with the parents, were evaluated for cold tolerance by determining the temperature that kills 50% of plants (LT_{50}) and analysed for protein accumulation by Western analysis (Fig. 4). Chromosome 5A of CNN conferred a significant ($P < 0.05$) increase in cold tolerance over that of the recipient (CS) parent (Fig. 4A). The chromosome 1A substitution was less cold tolerant than CS. Two replicates of these same chromosome substitution lines were evaluated for cold tolerance under natural cold-acclimating conditions in the field. Under field conditions chromosome substitutions 5A and 5D were equally the most cold tolerant, while substitution 1A was again the least cold tolerant line.

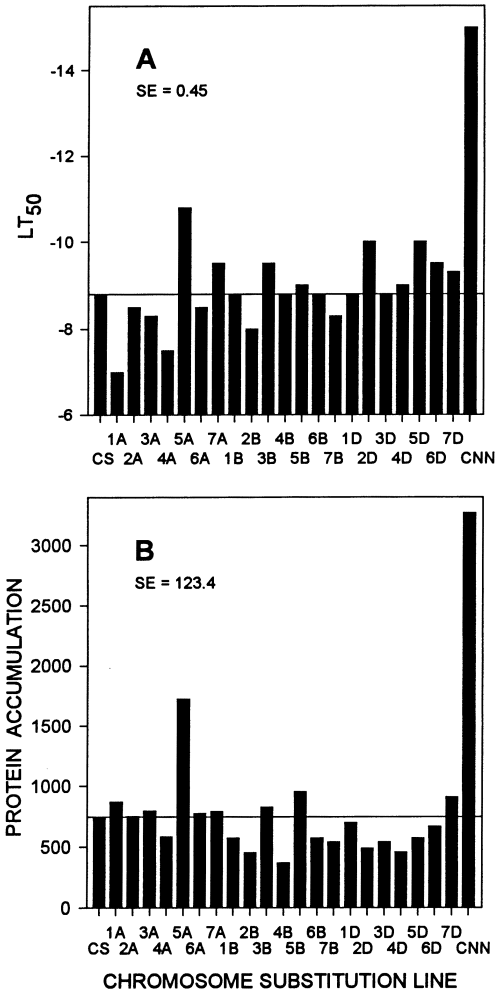

Fig. 4A, B *T. aestivum* cultivars Chines Spring (CS) and Cheyenne (CNN), together with chromosome substitution lines in which each designed chromosome pair of CS is replaced by its homologue from CNN. **A** Cold Tolerance (LT_{50}) of LT-acclimated lines. **B** Relative levels of WCS120 proteins determined by densitometry scanning of Western blots

As expected the more cold-tolerant parent CNN accumulated greater amounts of the WCS120 proteins than did the less tolerant CS parent. Densitometer scanning of Western blots of the CS/CNN substitution series (Fig. 4B) showed that only substitution of chromosome 5A of CNN for 5A of CS resulted in significantly ($P < 0.05$) greater protein accumulation than was found in CS or any of the other substitution lines.

Discussion

Chromosomal and genomic location of *Wcs120* family genes

Southern and Western analyses of the *Wcs120* gene family of hexaploid wheat localized these genes to the long arm of the group 6 chromosomes. Each of the three genomes (A, B, and D) of hexaploid wheat bears one or more members of this gene family. Diploid species carrying the A or D genomes, as well as the AB genome of tetraploid species were screened by Western analysis; the data confirmed the genomic assignments of the coding genes. Using high stringency conditions, hybridization intensities in Southern analysis of the A and D genomic species revealed the presence of only one band, considered to represent *Wcs120* or its homoeologues in the A and D genomes. Curiously, although all of the related species express a homoeologue to *Wcs120* whose product is identified by the WCS120 antibody, chromosome arm 6DL is the only region with a confirmed *Wcs120* locus and the only *Wcs120*-expressing locus in the hexaploid wheat CS.

Evolutionary relationships

In the three endonuclease digests (not all shown) of one D genome accession SQ44, all hybridizing fragments, including the *Wcs120* locus, could be matched to apparently identically sized fragments in the CS hexaploid. The similarly of the D genome accession SQ44 to the probable D genome in CS wheat revealed by Southern analysis may reflect the fact that the D genome was the most recent addition to hexaploid wheat and few changes have occurred since its incorporation into the polyploid. This result lends support to the evidence of Lagudah et al. (1991) that the D genome of *T. aestivum* comes from only a small population of the *T. tauschii* species. DNA sequence homology and WCS120 antibody recognition of the LT-induced proteins from this gene family all indicate a gene location on the long arm of the sixth group chromosomes and thus imply a homoeologous relationship. Although many chromosomal rearrangements have occurred during the evolution of species within the Triticeae group, Gill et al. (1993) found that 24 of 25 probes from chromosome 6D of *T. tauschii* also mapped to their sixth group homoeologues (6A, 6B, and 6D) in hexaploid wheat in the same linear order, indicating that homoeology and relative position is generally well conserved among the three genomes. Members of the related dehydrin family (*dhn3, dhn4,* and *dhn5*) in *Hordeum vulgare*, another species belonging to the Triticeae, have also been mapped to homoeologous chromosome 6 in barley (Close and Chandler 1990; Pan et al. 1994). One of the dehydrin genes (*dhn5*) has also been shown to be LT induced (van Zee et al. 1995). These group 6 chromosomes therefore appear to be the location of many structural genes induced by stress. Shared homology (Close et al. 1995; Welin et al. 1994) and chromosome location, along with some form of stress induction, suggest a common origin for these genes.

Polyploid gene expression

We have previously found (Limin et al. 1995) that when synthetic hexaploid wheat (ABD) is produced, by com-

bining an AB genome tetraploid wheat with the D genome of *T. tauschii*, the WCS120 protein homologues of both parents are expressed in the new synthetic hexaploid. Based on the data presented here, modern *T. aestivum* (CS) however, does not appear to be simply the result of genome addition to form the hexaploid. All A and D genome species examined possessed a 50 kDa protein, but the deletion of the long arm of the group 6 D genome chromosome (line 6DS in hexaploid wheat) resulted in the absence of a 50 kDa protein. In our Southern analysis (Fig. 3) hybridizing fragments with homology to the *Wcs120* probe were seen in both A and AB genome species. This suggests that the *Wcs120* homoeologue expressing the 50 kDa protein in the A genome and in the tetraploid AB genome species is suppressed in the CS polyploid.

Silencing of transgenes homologous to endogenous sequences in plants has received considerable attention recently (Flavell 1994) and it has been recognized that such cases may involve mechanisms that plants naturally use to modify gene expression (Matzke and Matzke 1995). In polyploid species, enzyme multiplicity, indicating expression of genes from more than one parent, is common but there is evidence for gene silencing of duplicate loci (Soltis and Soltis 1993). Gene silencing by a process such as methylation or some other means would provide a possible explanation for the inability of the A genome *Wcs120* homoeologue to be expressed in hexaploid CS wheat.

Gene regulation

Substitution of chromosomes from the more cold-tolerant cultivar Cheyenne (CNN) into less cold tolerant CS resulted in greater cold tolerance of lines 5A, 5D, and 2D, although only the 5A substitution was significantly more cold tolerant in this experiment. Several other chromosomes have been shown to endow CS with improved cold tolerance under various cold-hardening regimes, but chromosome 5A, followed closely by chromosome 5D (Sutka 1981), have almost invariably been observed to confer the greatest cold tolerance. Field-acclimated material, evaluated as soil temperatures were reaching freezing point, confirmed that the major genes of CNN conferring increased cold tolerance to CS were located on CNN chromosomes 5A and 5D. Both 5A and 5D substitutions were equal in cold tolerance and were 2° C more cold tolerant than any other substitution line, supporting the results of the growth chamber experiment and previous studies. This does not include all chromosome lines of this series that have been previously reported to improve cold tolerance. The finding that several CNN chromosomes influence cold tolerance in CS is indicative of a trait controlled by several genes. In this study no single chromosome substitution brought the cold tolerance of CS close to the CNN level (Fig. 4A). Accumulation of the protein products of the *Wcs120* gene family (Fig. 4B), as expected, was much greater in CNN than in CS during cold acclimation. In the chromosome substitution lines only the 5A substitution showed significantly greater protein accumulation than CS during cold acclimation. Since the genes expressing these proteins reside on the long arm of the group 6 chromosomes, regulation of the *Wcs120* gene family in this instance appears to be mediated by loci on chromosome 5A. Interestingly, this chromosome is most frequently found to have the greatest effect on cold tolerance, and the accumulation of proteins that cross-react with anti-WCS120 has also been associated with the development of cold tolerance (Houde et al. 1992a; Fowler et al. 1996a).

In barley Hayes et al. (1993) found that the largest quantitative trait loci (QTL) effects for winter field survival, LT_{50}, growth habit, and crown fructan content were located on the long arm of chromosome 7 (equivalent of group 5 in wheat). It may be that barley chromosome 7 acts in the same manner as wheat chromosome 5A in that it may influence growth habit and regulate the expression of LT-induced genes, such as *dhn5*, located on the same homoeologous group 6 chromosome arm as are the *Wcs120* family genes. RFLP and other marker-based evidence (Plaschke et al. 1993; Laurie et al. 1995) indicates that spring/winter growth habit determining genes *Vrn1, Vrn3,* and possibly *Vrn4* of wheat chromosomes 5A, 5D, and 5B, respectively, as well as *Sh2* of barley and *Sp1* of rye (*Secale cereale* L.), are a homoeoallelic set in the Triticeae. This would explain why the QTL effects were assigned to barley chromosome 7 and not to chromosome 6 where *dhn5* is located; although *dhn5* expression has not yet been associated with cold tolerance (van Zee et al. 1995), it was found to be LT specific.

Chromosomes 5A and 5D carry vernalization genes conditioning spring/winter growth habit in wheat. Fowler et al. (1996b) have observed that vernalization fulfilment (saturation) and loss of low-temperature tolerance are closely related. Down-regulation of *Wcs120* mRNAs and decline in protein accumulation were also closely associated with the point of vernalization saturation in wheat and rye (Fowler et al. 1996a). In wheat, 'spring habit' is dominant (Pugsley 1971) and 'winter habit' types carry recessive alleles at all vernalization (*vrn*) loci. Spring types may, however, still retain a vernalization (LT requirement) response that delays the reproductive phase. CNN carries recessive vernalization alleles at all *vrn* loci but CS carries a spring habit allele, *Vrn3*, on chromosome 5D (Pugsley 1972). The CS/CNN 5D chromosome substitution line may therefore influence cold tolerance by making the CS parent effectively into a winter type by replacing *Vrn3* with *vrn3*, thereby making all vernalization loci recessive. We have observed that this does lengthen the vegetative phase and thereby delay anthesis by approximately 40 days. This delay could allow greater accumulation of cold tolerance-conferring proteins (Limin et al. 1995; Fowler et al. 1996a). The 5A substitution does not have a significant effect on vernalization response, unlike the 5D sub-

stitution (Law et al. 1976), and therefore increased cold tolerance of that line is not likely to be due to differences in vernalization requirement. The WCS120 proteins, however, were not detectably influenced by the CNN 5D substitution in this experiment. Chromosome 5D (and its homoeologues) may also carry structural or regulatory genes affecting another cold tolerance-conferring mechanism, since substitution 5D is usually found to increase cold tolerance.

The increased accumulation of the WCS120 proteins in CS/CNN 5A substitution lines, with a concomitant increase in cold tolerance, implies that a gene located on chromosome 5A controls expression of the *Wcs120* gene family. This regulation is not active on the A genome (*Wcs200*) alone, but extends to the B genome (*Wcs66*) and the D genome (*Wcs120*). This suggests this group of genes has common promoter sites and transcriptional elements, although it is possible that the regulation may be indirect. Northern and Western analysis has previously revealed low-temperature induced *Wcs120* mRNAs and WCS120-like proteins in several related species (Houde et al. 1992a; Limin et al. 1995), indicating gene conservation within the Triticeae. Coordinate regulation of this gene family has also been observed within wheat cultivars (Houde et al. 1992a), suggesting a common system of regulatory control influencing all genomes in the hexaploid. If a gene on chromosome 5A is part of a direct regulatory system of the *Wcs120* gene family, this gene may have evolved as an independent regulator or may influence these structural genes as part of a competitively balanced system with regulatory loci on the group 5 chromosomes of all genomes.

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