Theor Appl Genet (1997) 95:265–270

G. Galiba · I. Kerepesi · J. W. Snape · J. Sutka

Location of a gene regulating cold-induced carbohydrate production on chromosome 5A of wheat

Received: 3 March 1997 / Accepted: 7 March 1997

Abstract Major changes in osmotic potential during cold acclimation are due to changes in sugar concentration, and there is a good correlation between sugar content and frost tolerance. The objective of the present study was to localize a gene(s) responsible for carbohydrate accumulation during cold acclimation on chromosome 5A of wheat using recombinant lines developed from the cross between the substitution lines Chinese Spring (Cheyenne 5A) and CS(*Triticum spelta* 5A). Previously, major genes influencing frost resistance (*Fr1*) and vernalization requirement (*Vrn1*) had been localized on the long arm of that chromosome. The *T. spelta* 5A chromosome carrying the *Fr1* (frost-sensitive) allele for frost tolerance and the *Vrn1* (spring-habit) allele for vernalization requirement did not have a major effect on the sucrose and fructan contents in the Chinese Spring background. On the other hand, the presence of Cheyenne alleles for vernalization requirement, *vrn1*, and frost tolerance, *fr1*, significantly increased sugar concentrations. A recombinant line thought to exhibit recombination between the *Vrn1* and *Fr1* loci suggested that the gene regulating sucrose accumulation was closely associated with, or else represented a pleiotropic effect of, *Vrn1*, but was separable from the *Fr1* locus.

Key words Carbohydrate · Frost resistance · Fructan · RFLP mapping · Sucrose · Wheat

Communicated by G. Wenzel

G. Galiba (✉) · J. Sutka
Agricultural Research Institute of the Hungarian Academy of Sciences, H-2462 Martonvasar, Hungary

I. Kerepesi
Department of Chemistry and Biochemistry, Janus Pannonius University, H-7601 Pecs, Hungary

J. W. Snape
John Innes Centre, Colney, Norwich NR4 7UJ, UK

Introduction

Winter exposes young wheat seedlings to many stresses including a direct effect of frost, ice-encasement, flooding, heaving, desiccation and various diseases usually known as snow moulds. However, tolerance to low freezing temperatures has been considered as the primary limiting factor for survival in most regions (McKersie and Leshem 1994). To achieve the full genetic potential of frost tolerance the plant must be hardened. Under natural conditions the cold hardening (acclimatization) takes place in autumn when the temperature gradually decreases to 0°C over several weeks. Temperatures of 2–5°C and photoperiods of about 12 h are considered to be optimal for cold hardening under controlled environmental conditions, although sublethal freezing temperatures may induce a second phase in the hardening process leading to the maximal expression of freezing tolerance (Fowler et al. 1983; Kacperska 1993).

Numerous reports have shown that the accumulation of various osmotically active solutes, such as sucrose, fructan, quaternary ammonium compounds and proline, takes place during hardening (Thomashow 1990; Galiba 1994; McKersie and Leshem 1994). These may function as cryoprotectants, helping to avoid cellular dehydration during extracellular ice formation, and many of these compounds may also stabilize membranes. Carbohydrate changes during hardening are of particular importance because of their direct relationship with such physiological processes as photosynthesis, translocation and respiration. Among the soluble carbohydrates, sucrose and fructants have some potential role in adaptation to cold stress (Housley and Pollock 1993; McKersie and Leshem 1994). Sucrose can act in water replacement to maintain membrane phospholipids in the liquid-crystalline phase and to prevent structural changes in soluble proteins.

In addition to acting as plant carbohydrate reserves, fructants may have other functions, including involvement in drought and frost tolerance in many grasses, e.g. wheat and barley. The accumulation of fructans is induced by unfavourable conditions, such as cold or drought, that reduce plant growth. Under these conditions, photosynthesis is less inhibited than growth, sucrose concentrations will increase (as a substrate for fructan synthesis), which may further enhance fructan biosynthesis (Housley and Pollock 1993). Recent studies of overwintering cereals have shown that freezing tolerance is strongly correlated with the capacity to increase photosynthesis and with the capacity to increase soluble carbohydrate pools during cold hardening (Tognetti et al. 1990; Öquist et al. 1993). Furthermore, field studies have shown that plants become vulnerable to freezing when the fructan pool becomes depleted and simple sugars can no longer be released into the cytosol and intracellular liquid (Olien and Clark 1993). Hurry et al. (1995) compared the carbon metabolism in spring and winter cultivars of wheat and oil-seed rape during long-term exposure to low temperature. They found increased enzyme activity (fructose-1,6-bisphosphatase and sucrose-phosphate synthase) only in the winter cultivars. This was associated with increased soluble/insoluble carbohydrate ratios in winter cultivars while spring wheats showed a relative decrease in soluble carbohydrates. Roberts (1993) studied the effect of continuous sucrose feeding on the cold hardiness of 'Kharkov 22 MC' wheat plants. His data suggested that increases in sugar content increase hardiness.

Certain chromosomes are known to carry genes that play an important role in the development of frost tolerance. For instance, if the 5A chromosome of the frost-sensitive genotype Chinese Spring (CS) is replaced by the corresponding chromosome from the winter variety Cheyenne (Ch), which possesses excellent frost tolerance, the frost tolerance of CS is greatly increased (Sutka 1981; Veisz and Sutka 1989). Major genes influencing frost tolerance (*Fr1*) and vernalization requirement (*Vrn1*) were mapped on the long arm of chromosome 5A (Roberts 1989; Sutka and Snape 1989; Galiba et al. 1995) using recombinant lines developed from the cross between the substitution lines CS(Ch 5A) and CS (*Triticum spelta* 5A) (Galiba et al. 1995). The data showed that although a close genetic linkage exists between the loci *Fr1* and *Vrn1*, they appeared to be separable. The location of *Vrn1* suggests that it is homoeologous to other vernalization genes in related species, particularly to the *Sh2* locus on chromosome 7(5H) of barley (Laurie et al. 1995) and to the *Sp1* locus on chromosome 5R of rye (Plaschke et al. 1993). An association between frost tolerance and fructan content was reported by Hayes et al. (1993) in barley, and QTLs controlling traits associated with winter hardiness (field survival, LT50, vernalization response and fructan content) have also been mapped to chromosome 7(5H).

In a preliminary experiment the elevated carbohydrate levels during cold stress of the CS(Ch) chromosome-substitution lines were studied. This showed that the Ch 5A chromosome raised total sugar, fructan and invertase activity as well frost tolerance in the CS background (Housley et al. 1993). These results prompted us to investigate in greater detail the sugar concentrations of CS, Ch and the 5A recombinant-substitution lines, and the results of these analyses are reported here.

Materials and methods

Genetic stocks

The eight recombinant lines studied here were developed from the cross between the substitution lines, CS (*T. spelta* 5A) and CS (Ch 5A), described above (Galiba et al. 1995).

Plant growth and cold treatment

Cold hardening was carried out using the method developed at Martonvasar (Sutka 1981). Briefly, the seedlings were grown in wooden boxes, and subjected to gradually decreasing temperature and illumination in a Conviron growth chamber for 5 weeks as follows: day and night temperatures, respectively, of 15°C and 10°C in the 1st week, 10°C and 5°C in the 2nd and 3rd weeks, 5°C and 0°C in the 4th and 5th weeks. The duration of the illumination was 12 h for 3 weeks, after which it was shortened to 8 h for the last 2 weeks. The photosynthetic photon flux during the growth of the plants was 260 $\mu mol\, m^{-2}\, s^{-1}$.

Chemical analysis

Water-soluble carbohydrate content was determined on fresh plant material (shoot bases including crown and leaf sheaths). Samples of 2 g fresh weight were extracted twice under reflux using 40 ml of boiling water for 15 min, and twice using 40 ml of 80% boiling ethanol for 15 min (Kerepesi et al. 1996). Fractions were collected and cleared by filtering through Whatman No. 42 papers. Filtrates were dried under reduced pressure at 40°C (vacuum evaporator Buchy model ASB, Sweden) and dissolved in distilled water. Oligosaccharides were hydrolysed by boiling in 0.5% HCl for 30 min. Amounts of free (analysed before hydrolysis) and bound (analysed after hydrolysis) glucose (Glu), fructose (Fru) and sucrose (Suc) were measured using Boehringer Mannheim GmbH Glu/Fru/Suc, No. 716 260 Kits (Wagner et al. 1983). Total amounts of water-soluble carbohydrates were determined using the phenol-sulphuric-acid method (Dubois et al. 1956).

Statistical analysis

Four replicate samples of each plant material were taken from each genotype. The data were analysed by the STATGRAPHICS statistical package, using the *t*-test and ANOVA functions, to assess significant differences between genotype and treatment means.

Results

Carbohydrate content in the stems of CS and Cheyenne

Control plants

Total water-soluble carbohydrate (WSC) contents were significantly lower ($P > 0.05$) in the non-hardened (NH) control plants of CS than in Ch (Table 1), which would be expected from their frost tolerance levels. The mono- and di-saccharide contents were similar, although the values were higher in Ch. The Fru concentration was approximately 2.5-times higher in the Ch shoots, whilst the glucan contents were similar.

Cold-hardened plants

Cold treatment increased the carbohydrate concentration considerably in both cultivars. The cold-hardened (CH) shoots of both parents showed approximately a 10-fold increase in WSC. Concentrations of all of the main sugar components (Glu, Fru and Suc) increased in both genotypes in the same manner, where Suc increased approximately 15-fold, while Glu and Fru concentrations increased approximately 4-fold and 5-fold (Table 1). The sugar contents in the NH winter wheat plants were higher than in the spring line, and this difference was reflected in CH plants where the Suc contents in Ch and CS were 43 and 15 mg/g F.Wt., respectively. Of the polysaccharides, the Fru contents of Ch and CS increased approximately 18- and 22-fold, respectively. Glu content increased 10-fold in Ch and 7-fold in CS following CH. Examination of the proportions of different sugar components of WSC suggests that CH increased the percentage of Suc and Fru and decreased the proportions of monosaccharides and Glu in both varieties. The proportion of Suc increased slightly from 6% to 8% in CS while Glu and the Fru decreased considerably from 40% to 19% and 17% to 12%, respectively, but still remained higher than the Suc level. The percentage of Fru increased from 19% to 46%. In Ch the incremental change of Suc from 8% to 13% during CH was greater than that of each monosaccharide. The Glu and Fru percentages decreased from 31% to 11% and 14% to 9%, respectively. The contribution of Fru to WSC increased from 29% to 50%. These changes show that the large CH-induced Fru and Suc accumulation is an adaptive response to cold. Moreover the concentrations of these sugars in Ch were considerably higher than in CS, independent of the growing conditions. These genetic differences between CS and Ch prompted the investigation of the Suc and Fru contents of the 5A recombinant-substitution lines.

Distribution of sugars in the recombinant lines

The presence of the *T. spelta Fr1* (frost-sensitive) allele and the *Vrn1* (spring-habit) allele for vernalization requirement (symbolised in Fig. 1 as "T") did not change

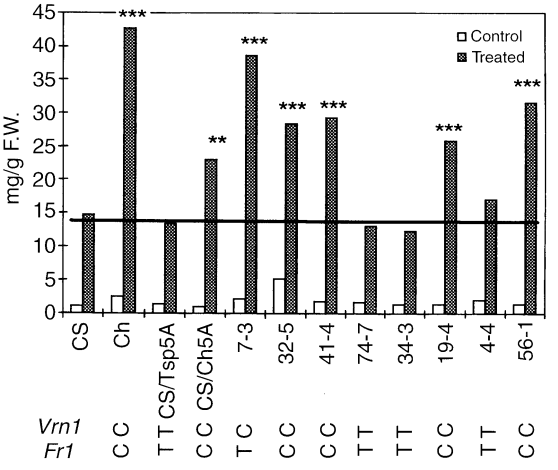

Fig. 1 Effect of cold hardening on sucrose concentration in stems of spring wheat 'Chinese Spring' (*CS*), winter wheat 'Cheyenne' (*Ch*), CS (*T. spelta* 5A) and CS (Ch 5A) substitution lines and eight of the single-chromosome recombinant lines developed from these 5A substitutions. The frost-tolerance (*Fr1*) and vernalization-requirement (*Vrn1*) genes representing *T. spelta* and Ch alleles are symbolised by *T* and *C*, respectively. Each column represents the mean of four stems. The differences from the mean of CS (*T. spelta* 5A), marked with a *horizontal line*, were calculated among the cold-hardened samples only. Significant differences were calculated at the $P < 0.05$ (*), $P < 0.01$ (**) and $P <$ (***) levels

Table 1 Carbohydrate content of non-hardened (NH) and cold-hardened (CH) stems of spring wheat Chinese Spring and winter wheat Cheyenne. Each value represents the mean ± SD of four stems

Carbohydrates	Carbohydrate content (mg/g fresh wt)			
	Chinese Spring		Cheyenne	
	NH	CH	NH	CH
Glucose	7.9 ± 0.6	33.4 ± 1.8	10.1 ± 1.0	36.3 ± 0.8
Fructose	3.3 ± 0.4	20.8 ± 1.3	4.6 ± 0.3	29.4 ± 0.7
Sucrose	1.1 ± 0.6	14.7 ± 2.0	2.5 ± 1.0	42.5 ± 3.0
Glucan	3.8 ± 2.0	27.3 ± 2.5	5.3 ± 2.9	51.7 ± 4.5
Fructan	3.7 ± 0.5	83.1 ± 10.7	9.3 ± 1.9	165.0 ± 7.8
Total WSC[a]	19.8 ± 4.3	179.3 ± 18.6	31.8 ± 6.9	324.9 ± 27.3

[a] WSC = water-soluble carbohydrate

the Suc content in the 'Chinese Spring' background (Fig. 1). On the other hand, the presence of the 'Cheyenne' allele (symbolised as "C") for vernalization requirement, *vrn1*, significantly increased the sucrose concentration. From the genotype of the recombinant between these loci, 7-3, the gene regulating sucrose accumulation seems to be closely associated with the *Vrn1* locus but separable from the *Fr1* locus. This was also the case with a gene affecting ABA accumulation (Quarrie et al. 1994 a).

The presence of the complete *T. spelta* 5A chromosome in the 'Chinese Spring' background decreased the rate of Fru accumulation during cold treatment (Fig. 2) whilst the 'Cheyenne' 5A increased the Fru content. From the genotype of the recombinant line, 7-3, the *Vrn1* locus again appears linked to the gene(s) controlling stress-induced fructan accumulation. However, the effect is less pronounced and variation between the lines may indicate that another gene(s) on 5A may be segregating for Fru accumulation independent of *Vrn1*.

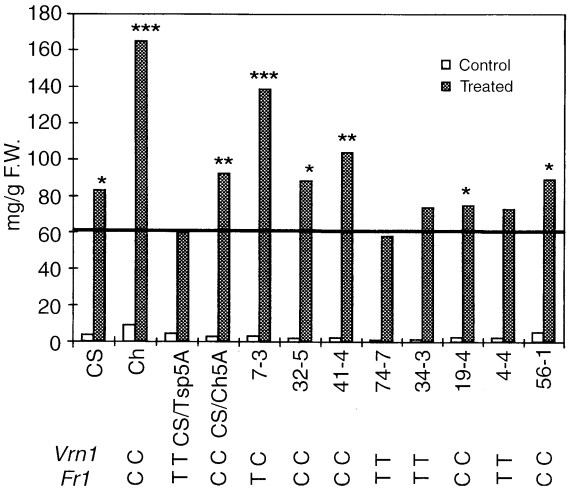

Fig. 2 Effect of cold hardening on fructan concentration in stems of spring wheat 'Chinese Spring' (*CS*), winter wheat 'Cheyenne' (*Ch*), CS (*T. spelta* 5A) and CS (Ch 5A) substitution lines and eight of the single-chromosome recombinant lines developed from these 5A substitution. Otherwise, as in Fig. 1

Discussion

Measurements of sugar concentrations on the 5A recombinant-substitution lines (Table 2, Figs. 1 and 2) clearly shows that there is close genetic linkage between the genes responsible for cold-induced sugar accumulation and the *Fr1* and *Vrn1* loci. Although several papers have indicated a positive correlation between sugar accumulation and frost tolerance, this is the first report which represents data on the close genetic linkage between the major genes influencing winter hardiness and the genes that regulate cold-induced sugar production. Based on this result the monitoring of Suc or Fru contents in breeding programs could be used as a selection criterion to achieve better winter hardiness.

The presence of three known RFLP loci in this region of chromosome 5A (Galiba et al. 1995) may make this region amenable for gene isolation and cloning, following the recent mapping results of Gill et al. (1996). For chromosome 5A they found that more than 60% of the markers (marker distribution may represent the distribution of genes) were present in three major clusters that physically encompassed less than 18% of the long arm of chromosome 5A. According to their map, the *Fr1* and *Vrn1* genes are flanked by two probes

Table 2 The genomic composition of the single-chromosome recombinant lines developed from the intercross of CS (*T. spelta* 5A) and CS (Ch 5A) substitution lines. The RFLP probes representing *T. spelta* and Ch alleles are symbolised by T and C, respectively

Distance (cM)	Marker	T.sp5A 55-6	Ch 5A 67-3	Single-chromosome recombinants						
				7-3	32-5	41-4	74-7 34-3	19-4	4-4	56-1
0	*psrB85*	T	C	T	T	C	T	C	C	C
	psrB85	T	C	T	T	C	T	C	C	C
6.4	*psr911*	T	C	T	T	C	T	C	C	C
15	*psr637*	T	C	T	T	C	T	C	C	C
56.7	*Xpsr2021* (*dhn2*)	T	C	C	T	C	T	C	T	C
65.4	***Vrn1***	**T**	**C**	**C**	**C**	**C**	**T**	**C**	**T**	**C**
	Xcdo504	T	C	C	C	C	T	C	T	C
	Xwg644	T	C	C	C	C	T	C	T	C
	Xpsr426	T	C	C	C	C	T	C	T	C
67.5	***Fr1***	**T**	**C**	**T**	**C**	**C**	**T**	**C**	**T**	**C**
113.1	*Xpsr805* (*Embp*)	T	C	T	T	T	C	T	C	T
117.7	*Q*	T	C	T	T	T	C	T	C	T
122	*Xpsr370*	T	C	T	T	T	C	T	C	T
5A/4A break point										
128.4	*Xpsr164*	T	C	T	T	T	C	T	C	T
178.9	*B-amy-A1*	T	C	T	T	C	T	C	C	T

mapped in the gene cluster at FL 0.75. In this particular submicroscopic region ten other probes were mapped. The bp/cM estimates for this region may be anywhere from 40 to 120 kb/cM, which is similar to other crop plants possessing smaller genomes like tomato (Ganal et al. 1989; Zhang et al. 1994) or rice (Umehara et al. 1995).

It is not clear how carbohydrate biosynthesis is regulated during cold hardening and what is the role of the genes on chromosome 5A. Housley and Pollock (1993) pointed out that there are at least six possible sites for the regulation of carbohydrate metabolism in leaf cells of temperate grasses. In winter rye, in parallel with the increase in photosynthetic capacity following cold hardening, there is an increase in ribulose-1,5-bisphosphate carboxylase/oxygenase and sucrose-phosphate synthase activity and 3- to 4-fold increases in the pools of associated metabolites (Hurry et al. 1994). The regulation of fructan metabolism is based on its possible function, namely the control of sucrose content within the vacuole (Housley and Pollock 1993). Understanding the control of sucrose content is important because of its major role in higher-plant carbohydrate partitioning. It is also believed to be responsible for changes in gene expression including the induction of fructan metabolic enzymes (Housley and Pollock 1985, 1993; Olien and Clark 1993). These findings support the proposal of Guy et al. (1992) that the increase in the activity and amount of enzymes of the photosynthetic carbon reduction cycle and of those responsible for Suc synthesis is not simply an adjustment for overcoming the slower enzyme kinetics at low temperature.

A polygenic regulation pattern might be required for the control of these rather complicated processes. The region around the *Vrn1* and *Fr1* genes may therefore contain a complex of regulatory genes. Dubcovsky et al. (1995) investigated the linkage relationships among genes responding to water-deficit, salt stress and heat shock in diploid wheat (*Triticum monococcum* L). They mapped a relatively high number of genes (two dehydrin and four early salt-induced (*Esi*) genes and a duplicate heat-shock locus *Xttu1934*(*Hsp16.9b*), which co-segregated with the *Xwg644* RFLP locus, in a 54.6-cM region on the telomeric end of chromosome $5A^mL$. Moreover a QTL for drought-induced ABA accumulation was also found in this region (Galiba et al. 1994; Quarrie et al. 1994a, b). Clearly much more detailed genetic information is required by further fine mapping of this region.

Acknowledgements These studies were supported by the Hungarian National Research Fund Nos. T016972 and T21118.

References

Dubcovsky J, Luo M-C, Dvorak J (1995) Linkage relationships among stress-induced genes in wheat. Theor Appl Genet 91:795–801

Dubois M, Gilles KA, Hamilton JK, Rebes PA, Smith F (1956) Colorimetric method for determination of sugars and related substrates. Anal Chem 28:350–356

Fowler DB, Limin AE, Gusta LV (1983) Breeding for winter hardiness in wheat. In: Fowler DB, Gusta LV, Sinklard AE, Hobin BA (eds) New frontiers of winter wheat production. University of Saskatchewan, Saskatoon, Canada, pp 136–184

Galiba G (1994) *In vitro* adaptation for drought and cold hardiness in wheat. In: Janick J (ed) Plant breeding reviews. John Wiley and Sons Inc, vol. 12, pp 115–162

Galiba G, Sutka J, Snape JW, Tuberosa R, Quarrie SA, Sarkadi L, Veisz O (1994) The association of frost tolerance gene *Fr1* with stress-induced osmolyte and abscisic and accumulation in wheat: In: Dörffling K, Brettschneider B, Tantau H, Pithan K (eds) Crop adaptation to cool climates. COST 814 Workshop, October 12–14, Hamburg, Germany, European Commission, pp 389–401

Galiba G, Quarrie SA, Sutka J, Morgounov A, Snape JW (1995) RFLP mapping of the vernalization (*Vrn1*) and frost-tolerance (*Fr1*) genes on chromosomes 5A of wheat. Theor Appl Genet 90:1174–1179

Ganal MW, Young ND, Tanksley SD (1989) Pulsed-field gel electrophoresis and Physical mapping of large DNA fragments in the *Tm-2a* region of chromosome 9 in tomato. Mol Gen Genet 215:395–400

Gill KS, Gill BS, Endo TR, Boyko EV (1996) Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics 143:1001–1012

Guy CL, Hubert JLA, Hubert SC (1992) Sucrose phosphate synthase and sucrose accumulation at low temperature. Plant Physiol 100:502–508

Hayes PM, Blake T, Chen THH, Tragoonrung S, Chen S, Pan A, Liu B (1993) Quantitative trait loci on barley (*Hordeum vulgare* L.) chromosome 7 associated with components of winter hardiness. Genome 36:66–71

Housley TL, Pollock CJ (1985) Photosynthesis and carbohydrate metabolism in detached leaves of *Lolium temulentum* L. New Phytol 99:483–499n

Housley TL, Pollock CJ (1993) The metabolism of fructan in higher plants. In: Suzuki M, Chatterton NJ (eds) Science and technology of fructans. CRC Press, Boca Raton, pp 191–225

Housley TL, Seibert AC, Galiba G, Sutka J (1993) Sugar metabolism in cold-acclimated tissues of wheat chromosome 5 substitution lines. American Society of Agronomy, Agronomy abstract, Nov 7–12, Cincinnati, Ohio, pp 74–77

Hurry VM, Malberg G, Gardeström P, Öquist G (1994) Effects of short-term shift to low temperature and of long-term cold hardening on photosynthesis and ribulose-1,5-bisphosphate carboxylase/oxygenase and sucrose phosphate synthase activity in leaves of winter rye (*Secale cereale* L.). Plant Physiol 106:983–990

Hurry VM, Strand A, Tobiaeson M, Gardeström P, Öquist G (1995) Cold hardening of spring and winter wheat and rape results in differential effects on growth, carbon metabolism, and carbohydrate content. Plant Physiol 109:697–706

Kacperska A (1993) Water potential alterations – a prerequisite or a triggering stimulus for the development of freezing tolerance in overwintering herbaceous plants? In: Li PH, Christersson L (eds) Advances in plant cold hardiness. CRC Press Inc, London, pp 73–91

Kerepesi I, Tóth M, Boross L (1996) Water-soluble carbohydrates in dried plant. J Agric Food Chem 44:3235–3239

Laurie DA, Pratchett N, Bezant J, Snape JW (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

McKersie BD, Leshem YY (1994) Stress and stress coping in cultivated plants. Kluwer Academic Publishers, Dordrecht

Olien CR, Clark JL (1993) Changes in soluble carbohydrate composition of barley, wheat, and rye during winter. Corp Sci 85:21–29

Öquist G, Hurry VM, Huner NPA (1993) Low-temperature effects on photosynthesis and correlation with freezing tolerance in spring and winter cultivars of wheat and rye. Plant Physiol 101 : 245–250

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

Quarrie SA, Galiba G, Sutka J, Snape JW, Semikhodski A, Steed A, Gulli M, Calestani C (1994 a) Association of a major vernalization gene of wheat with stress-induced abscisic acid production. In: Dörffling K, Brettschneider B, Tantau H, Pithan K (eds) Crop adaptation to cool climates, COST 814 Workshop, October 12–14, Hamburg, Germany, European Commission, pp 403–414

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

Roberts DWA (1989) Identification of loci on chromosome 5A of wheat involved in control of cold hardiness, vernalization, leaf length, rosette growth habit, and height of hardened plants. Genome 33 : 247–259

Roberts DWA (1993) Studies of winter hardiness and related processes in wheat. Bulletin, Lethbridge Research Station

Sutka J (1981) Genetic studies of frost resistance in wheat. Theor Appl Genet 59 : 45–152

Sutka J, Snape JW (1978) Location of a gene for frost resistance on chromosome 5A of wheat. Euphytica 42 : 41–44

Thomashow MF (1990) Molecular genetics of cold acclimation in higher plants. Adv Genet 28 : 99–131

Tognetti JA, Salerno GL, Crespi MD, Pontis HG (1990) Sucrose and fructan metabolism of different wheat cultivars at chilling temperatures. Physiol Plant 78 : 554–559

Umehara Y, Inagaki A, Tanoue H, Yasukochi Y, Nagamura (1995) Construction and characterization of a rice YAC library for physical mapping. Mol Breed 1 : 79–89

Veisz O, Sutka J (1989) The relationships of hardening period and the expression of frost resistance in chromosome substitution lines of wheat. Euphytica 43 : 41–45

Wagner W, Keller F, Wiemken A (1983) Fructan metabolism in cereals: induction in leaves and compartmentation in protoplast and vacuoles. Z Pflanzenphysiol 112 : 359–372

Zhang HB, Martin GB, Tanksley SD, Wing RA (1994) Map-based cloning in crop plants: tomato as a model system. II. Isolation and characterization of a set of overlapping yeast artificial chromosomes encompassing the jointless locus. Mol Gen Genet 244 : 613–621