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The relationship between vernalization requirement and frost tolerance in substitution lines of wheat

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Abstract

Two sets of wheat (*Triticum aestivum* L.) substitution lines for the homoeologous group 5 chromosomes, 5A, 5B and 5D, carrying vernalization genes (*Vrn-A1, Vrn-B1, Vrn-D1*) were used to study the relationship between vernalization requirement and winter survival, with respect to the induction and maintenance of frost tolerance. Substitution lines carrying dominant *Vrn* loci substituted from the spring cultivars Zlatka (5A), Chinese Spring (5D) and the alternative cultivar Česká Přesívka (5B) into three different winter wheat backgrounds, Vala, Košútka and Zdar, showed lower winter survival by 20, 36, and 41 % for substitutions of 5B, 5A and 5D, respectively, compared to the original winter cultivars. Reciprocal substitution lines between two winter cultivars Mironovskaya 808 and Bezostaya 1 carrying different recessive alleles, *vrn-A1, vrn-B1, vrn-D1*, did not exhibit a modified induction of frost tolerance, but the duration of good frost tolerance, as well as the ability to survive the whole winter, was changed. In accordance with the model suggesting that genes for vernalization act as a master switch regulating the duration of frost tolerance, substitutions of homoeologous group 5 chromosomes induced, at first, frost tolerance at a level equal to the parental cultivar, and then, relative to the different extent of saturation of vernalization requirement, they gradually lost both frost tolerance and their ability to re-induce significant frost tolerance with a drop in temperature following warm periods in the winter.

Additional key words: growth habit, heading, winter survival, freezing, group 5 chromosomes, *Triticum aestivum* L.

Introduction

Frost tolerance (FT) of wheat, a very important component of winter hardiness, changes during the course of the winter. Generally, three FT stages can be characterized (Gusta and Fowler 1979, Prášil *et al.* 1994): *1.* hardening (acclimation) in autumn, when FT rises because of a slow decrease in temperature; *2.* maintenance of FT, which is characterized by a fluctuation of FT dependant on internal and external factors; *3.* dehardening – a gradual loss of FT due to a resumption of plant development after warming in spring.

The wheat plant ability to harden and maintain FT decreases after the transition from the vegetative to the reproductive phase (Prášil *et al.* 1994, Mahfoozi *et al.* 2001a,b). Winter wheat has developed mechanisms that extend the vegetative phase, vernalization requirement (the necessity of going through a certain period of low temperatures) and photoperiod sensitivity (day length sensitivity). Fowler *et al.* (1996, 1999) found out that any factor that delays the transition from the vegetative to generative phases produces increased FT. Recently Limin and Fowler (2002) and Danyluk *et al.* (2003) have concluded that genes for vernalization requirement act as a master switch regulating the duration of low temperature induced FT. In winter wheat, due to a longer vegetative phase, the FT is maintained for a longer time and at a higher level than in spring wheat. Fully vernalized winter wheat induced only a low level of FT as characteristic of spring wheat (Gusta *et al.* 1997, Mahfoozi *et al*. 2001a, b, Prášil *et al*. 2004).

Vernalization requirement is controlled by the *Vrn*

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Abbreviations: *Fr* - frost resistance genes; FT - frost tolerance, LT50 - lethal temperature, *Vrn* - vernalization genes.

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genes (Snape *et al.* 2001). Three main loci are involved in vernalization requirement, *Vrn-A1, Vrn-B1* a *Vrn-D1*, located on chromosomes 5A, 5B and 5D; in spring wheat a dominant *Vrn* allele is present, while in winter wheat all three loci have recessive alleles. Roberts (1990), Košner and Pánková (1998) showed that different vernalization requirements of winter wheat cultivars are the result of multiple alleles at *Vrn* loci. Molecular genetic studies have shown a very close genetic linkage between the vernalization and frost tolerance (*Fr*) genes (Galiba *et al.* 1995, Sutka 2001). The frost tolerance genes *Fr-A1, Fr-B1* and *Fr-D1* were located on chromosomes 5A, 5B and 5D in the regions where the highest concentration of QTLs and major loci controlling wheat resistance to abiotic stresses has been found (Cattivelli *et al.* 2002, Tóth *et al.* 2003).

Wheat chromosome substitutions of homoeologous group 5 have provided a useful tool for studying the genetic control of FT as well as of vernalization requirement (Cahalan and Law 1979, Sutka *et al.* 1986, Sutka 2001, Roberts 1990, Snape *et al.* 2001). Novel wheat substitution lines of the homoeologous group 5 chromosomes carrying dominant *Vrn* loci from spring to winter cultivars, and reciprocal substitution lines between two winter cultivars carrying recessive *vrn* alleles, were produced to study the effects of the group 5 chromosomes on vernalization requirement and on the growth stages and agronomic characters of wheat (Košner and Pánková 1998, 1999, 2001a,b). In this paper we describe studies of the relationship between vernalization requirement and winter survival, induction and maintenance of FT in wheat, using these substitution lines.

Materials and methods

Plants: Wheat (*Triticum aestivum* L.) lines with substituted homoeologous group 5 chromosomes were obtained by a series of backcrosses with monosomic lines (details and more information about the substitution lines are in Košner and Pánková 1998, 1999, 2001a,b). Two sets of the substitution lines after 5 to 6 backcrosses were used in this project. The first set of substitution lines carried dominant loci at *Vrn-A1, Vrn-D1 and Vrn-B1* from the spring cultivars Zlatka (ZL), Chinese Spring (CS) and the alternative cultivar Česká Přesívka (CP), respectively, in three different winter wheat backgrounds, Vala, Košútka and Zdar. These spring habit lines were Vala (ZL 5A), Vala (CP 5B), Vala (CS 5D), Košútka (ZL 5A), Košútka (CP 5B), Košútka (CS 5D), Zdar (ZL 5A), Zdar (CP 5B) and Zdar (CS 5D). The second set of substitution lines carried recessive *vrn* alleles having different vernalization requirements between two winter cultivars Mironovskaya 808 (MIR) and Bezostaya 1 (BEZ), and consisted of the reciprocal lines: MIR (BEZ 5A), MIR (BEZ 5B), MIR (BEZ 5D), BEZ (MIR 5A), BEZ (MIR 5B) and BEZ (MIR 5D).

Vernalization: Seeds of the substitution lines and the parental cultivars were germinated at one-week intervals and vernalized under 8-h photoperiod and temperatures from 1 to 3 °C, so that treatments of 8, 7, 6, 5, 4 and 3 weeks of vernalization were sown in field plots on the 20th April each year. At this time the photoperiod at the geographic latitude of the experiment (50° N) exceeded 14 h, and after this date no substantial additional vernalization due to low temperatures occurred (Košner and Pánková 1998). Time to heading was monitored for 10 plants of each treatment and substitution line when half of the ear on the first tiller of a plant was showing. The vernalization requirement was established as the number of days sufficient for full vernalization, *i.e.* that heading time was not significantly decreased with further vernalization (Košner and Pánková 1998).

Winter-survival of the substitution lines and cultivars was assessed by a pot-culture method under natural conditions that was described in detail in the paper by Prášil and Rogalewicz (1989). Plants were grown in wooden boxes (40 × 30 × 15 cm) filled with soil and placed on the ground for the whole winter. In spring, plant survival as a percentage score was assessed for 60 plants of each line. Under this experimental regime the winter survival was affected principally by frost. Winter survival was tested over four winters (1999 to 2003). The conditions were severer in the latter two winters than during the first two, so that the majority of spring lines completely perished through these hard winters. The results of winter survival from two years (1999 to 2001) are presented for the first set of substitutions lines.

Frost tolerance of plants was evaluated by a laboratory freezing test. After 4d germination of wheat seeds at 20 °C, the young seedlings were cultivated in an aerated Hoagland 3 nutrient solution (including microelements) at 17 °C and with a 12-h photoperiod (irradiance of 400 μmol m^{-2} s^{-1}) (Faltusová-Kadlecová *et al.* 2002). At 15th day (three-leaf stage), they were exposed to cold acclimation under the same cultivation conditions except for the temperature being 2 °C. After 3-week or 10-week acclimation, the plants of each substitution line and cultivar were divided into groups of 8 to 10 plants and exposed to -4 °C for 20 h. Then, the groups of each treatment were exposed to 6 different frost intensities in separate freezers for 24 h. Frost temperatures differed by 2 °C and the rate of cooling and thawing was 2 °C h^{-1}. After thawing, the plants were cut down to 2.5 cm above the crown, the roots were submerged in a dish of water, and they were placed in a greenhouse with a temperature of 20 °C. After 5 to 6 d, the number of living and regenerating plants was analyzed for each freezing temperature. FT was expressed in LT50 values (lethal temperature at which 50 % of the plants are killed in a controlled-frost test), calculated according to the model of Janáček and Prášil (1991) on the basis of survival values of plants from several freezing temperatures.

Statistical evaluation was carried out on the basis of a multiple range test (LSD at the 5 % level) of averages calculated from 3 repetitions (*Unistat version 5.1*, *Unistat Ltd.*, London, UK).

Results and discussion

Substitution lines with dominant *Vrn* loci: Substitutions of the chromosomes originating from spring or alternative cultivars (Zlatka, Chinese Spring or Česká Přesívka) into three winter cultivars (Vala, Košútka or Zdar) changed the growth habit of plants from winter to spring type due to the presence of a dominant *Vrn* allele (Košner and Pánková 2001b). Their survival over winter significantly decreased compared to the parental winter cultivar (Fig. 1). Nevertheless, the winter survival was decreased by 20, 36, and 41 % for substitutions of chromosome 5B from Česká Přesívka, 5A from Zlatka and 5D from Chinese Spring, respectively, indicating differences between effects of the individual homoeologous group 5 chromosomes on the resistance to winter frost.

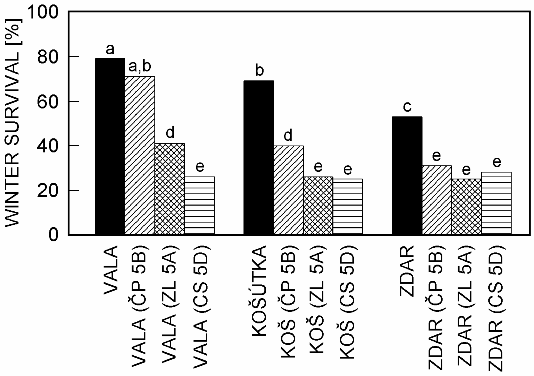

Fig. 1. Winter survival of substitution lines of homoeologous group 5 chromosomes (5A, 5B or 5D) from spring cultivars ZLATKA (ZL), CHINESE SPRING (CS) and alternative cultivar ČESKÁ PŘESÍVKA (ČP) into three different winter wheat backgrounds VALA, KOŠÚTKA (KOŠ) and ZDAR. Values denoted with the same letter are not significantly different.

The lines with a substituted 5B chromosome also showed the longest time to heading. On the other hand, the *Vrn-A1* locus appeared to be epistatic in reducing the vernalization requirement (Košner and Pánková 2001b, Snape *et al.* 2001). A smaller decrease of winter survival for the substitution of chromosome 5B from alternative cultivar Česká Přesívka could be explained by the presence of the weakest inhibition of vernalization by *Vrn-B1* on chromosome 5B, while the most considerable drop of winter survival of the lines with a substituted 5A chromosome corresponded to the strong effect of *Vrn-A1* (Košner and Pánková 2001b).

Studies using chromosome substitution lines of wheat indicate that the homoeologous group 5 chromosomes also carry major genes controlling FT as well as winter survival in wheat (Sutka 2001, Snape *et al.* 2001). Substitutions of these chromosomes into spring cultivars with the corresponding pair of chromosomes (*i.e.* 5A, 5B or 5D) from winter cultivars have usually produced lines with later flowering time and higher FT than the parental spring cultivar (Sutka 2001). A decrease of the vernalization response as well as of FT for the substitution of chromosome 5A from spring cultivar Rescue into winter cultivars Winalta or Kharkov has already been described by Roberts and MacDonald (1984, 1988). Here, we found that substitutions of homoeologous group 5 chromosomes with dominant *Vrn* loci to winter backgrounds changed plant growth habit and differentially decreased their resistance to winter frost probably due to different effects of *Vrn* and/or *Fr* alleles.

Reciprocal substitution lines with recessive *vrn* loci: Substitution lines with reciprocal substitutions of the homoeologous group 5 chromosomes containing recessive *vrn* loci between two winter cultivars Mironovskaya 808 (MIR) and Bezostaya 1 (BEZ) were studied for the effects of these chromosomes on growth and developmental characteristics (Košner and Pánková 1998, 1999, 2001a), and winter and frost tolerance (Fig. 2). Higher vernalization requirement and winter survival was observed in MIR than in BEZ. Substitution lines carrying chromosomes 5A and 5D of BEZ as well as the line carrying chromosome 5B of MIR showed lower vernalization requirement and winter survival than the parental cultivar. Košner and Pánková (1998) explained the different vernalization requirements by the dispersion of extreme alleles increasing (*vrn-A1* and *vrn-D1* from MIR and *vrn-B1* from BEZ) or decreasing (the reciprocal alleles) vernalization requirement. Then, the original cultivar MIR with higher vernalization requirement carries two alleles (MIR *vrn-A1* and *vrn-D1*) that increase the vernalization requirement, whereas BEZ with lower vernalization requirement only carries one (BEZ *vrn-B1*). The line MIR (BEZ 5B) with all of the three alleles increasing vernalization requirement had the highest and the line BEZ (MIR 5B) had the shortest vernalization requirement. The differences between the winter survival among the set of lines could be explained on the same basis because the correlation coefficient between the vernalization requirement and winter survival is high and significant (0.87). On the other hand, there are some differences in the detail, for instance MIR (BEZ 5A) with a lower vernalization requirement did not have a lower winter survival than the original MIR (Fig. 2). Sutka and Kovács (1985) observed a higher FT in both substitution lines for chromosome 5A between two winter wheat cultivars, Mironovskaya 808 and Rannyaya 12, than in the original cultivars. Finally, recent molecular analysis (Vágújfalvi *et al.* 2000, 2003) has predicted two loci for vernalization on chromosome 5A, which makes the explanation of a pleiotropic effect of *Vrn* genes on winter frost resistance more difficult.

Overwintering wheat plants go through three main stages of FT: acclimation, maintenance and deacclimation. The same dynamics of FT was observed under controlled cold acclimation when, at first, FT was gradually increased and after some time (6 to 11 weeks) it gradually decreased (Roberts 1979, Veisz and Sutka 1989, Fowler *et al.* 1996). Mahfoozi *et al.* (2001a,b) and Prášil *et al.* (2004) found that the FT decrease followed the saturation of vernalization requirement in winter wheat. Therefore, we measured FT of the substitution lines at the 3rd and 10th week of acclimation under controlled conditions, *i.e.* before and after the saturation of vernalization requirement of all substitution lines (Fig. 2). At the 3rd week of acclimation the differences in LT50s among MIR and the lines with MIR background and among BEZ and the lines with BEZ background were not significant; MIR was more resistant than BEZ. At the 10th week of acclimation the lines MIR (BEZ 5D) and BEZ (MIR 5B) with the lowest vernalization requirements showed a significantly lower FT than their parental lines. We conclude that the reciprocal substitutions for homoeologous group 5 chromosomes carrying recessive *vrn* loci between the two winter wheat cultivars did not influence induction of FT but influenced significantly the duration of high FT as well as the ability of wheat lines to survive the whole winter.

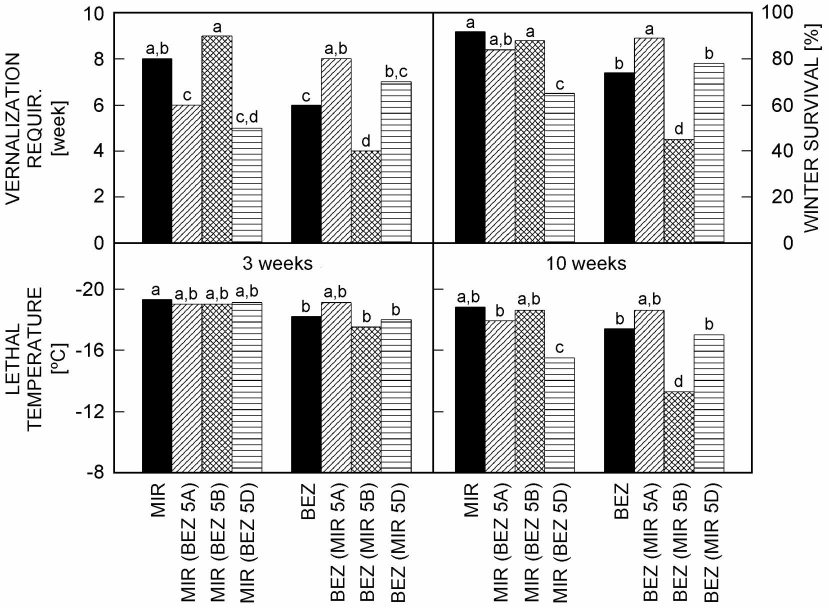

Fig. 2. Vernalization requirement, winter survival and lethal temperature after 3 and 10 weeks of cold acclimation of reciprocal substitution lines for homoeologous group 5 chromosomes (5A, 5B or 5D) between two winter cultivars Mironovskaya 808 (MIR) and Bezostaya 1 (BEZ). Values denoted with the same letter are not significantly different.

Vernalization requirement *versus* frost tolerance: A significant positive correlation between the duration of vernalization and resistance to winter was shown for a collection of West-European wheat cultivars by Schmütz (1976). A similar correlation was not proved for a collection of winter wheat cultivated in the Czech Republic during the second half of the 20th century (Petr and Hnilička 2002) or for cultivars of the world winter wheat collection of the Genebank in our institute (Prášilová, unpublished). A strong relationship was detected between growth habit and winter survival or FT (Roberts 1990, Braun 1997). On the other hand, it was possible to find winter wheat cultivars with a long vernalization requirement but moderate or low FT and vice versa (Gusta *et al.* 1997). Winter wheat plants are able to develop a high FT after exposure to cold acclimation but after fulfilment of vernalization requirement, in spring they develop only a low FT at the level of spring wheat (Gusta and Fowler 1979, Prášil *et al.* 1994, Fowler *et al.* 1999). Most winter wheat cultivars are fully vernalized in the field before the end of winter (usually before February) in the northern hemisphere. However, they only slowly loose FT due to a short photoperiod and a cold-reduced growth and development of plants (Prášil *et al.* 1994). In the spring, once temperatures increase, the start of active growth and development leads to a permanent loss of a high FT in winter wheat plants.

The ability of wheat to induce and maintain FT decreases after the vegetative to reproductive transition (Mahfoozi *et al.* 2001a,b). Fowler *et al.* (1999) and Danyluk *et al.* (2003) supported the model that the developmental genes (vernalization, photoperiod) that control the transition of plants from the vegetative to reproductive phase, also act as control genes for the duration of the expression of structural low-temperature-genes inducing FT. A vernalization requirement makes it possible for winter crops to maintain low-temperature-genes (COR, LTI, WCS, *etc.*) up-regulated for a longer period of time than those of spring crops. They concluded that any factor delaying the transition from the vegetative to generative phase produces increased FT. According to this model the substitution lines of the homoeologous group 5 chromosomes first induced FT at the level of the parental cultivar and then, relative to different lengths of saturation of vernalization requirement, they gradually lost their FT and their ability to re-induce sufficient FT following a drop in temperature after warm periods over the winter (Figs. 1, 2).

References

Braun, H.-J.: Winter hardiness of bread wheats derived from spring × winter crosses. - Acta agron. hung. **45**: 317-327, 1997.

Cahalan, C., Law, C.N.: The genetical control of cold resistance and vernalisation requirement in wheat. - Heredity **42**: 125-132, 1979.

Cattivelli, L., Baldi, P., Crosatti, C., Di Fonzo, N., Faccioli, P., Grossi, M., Mastrangelo, A.M., Pecchioni, N., Stanca, A.M.: Chromosome regions and stress-related sequences involved in resistance to abiotic stress in *Triticeae*. - Plant mol. Biol. **48**: 649-665, 2002.

Danyluk, J., Kane, N.A., Breton, G., Limin, A.E., Fowler, D.B., Sarhan, F.: TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. - Plant Physiol. **132**: 1849-1860, 2003.

Faltusová-Kadlecová, Z., Faltus, M., Prášil, I.: Comparison of barely response to short-term cold or dehydration. - Biol. Plant. **45**: 637-639, 2002.

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

Fowler, D.B., Limin, A.E., Ritchie, J.T.: Low-temperature tolerance in cereals: Model and genetic interpretation. - Crop Sci. **39**: 626-633, 1999.

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

Gusta, L.V., Fowler, D.B.: Cold resistance and injury in winter cereals. - In: Mussel, H., Staples, R.C. (ed.): Stress Physiology in Crop Plants. Pp. 160-178. John Wiley and Sons, New York 1979.

Gusta, L.V., Willen, R., Fu, A., Robertson, A.J., Wu, G.H.: Genetic and environmental control of winter survival of winter cereals. - Acta agron. hung. **45**: 231-240, 1997.

Janáček, J., Prášil, I.: Quantification of plant frost injury by nonlinear fitting of an s-shaped function. - Cryo-Letters **12**: 47-52, 1991.

Košner, J., Pánková, K.: The detection of allelic variants at the recessive vrn loci of winter wheat. - Euphytica **101**: 9-16, 1998.

Košner, J., Pánková, K.: Impact of homoeologous group 5 chromosomes with different vrn loci on leaf size and tillering. - Czech J. Genet. Plant Breed. **35**: 65-72, 1999.

Košner, J., Pánková, K.: The effect of the homoeologous group 5 chromosomes with different vrn loci on growth phases and quantitative characters of wheat. - Euphytica **119**: 289-299, 2001a.

Košner, J., Pánková, K.: Substitution lines of wheat with dominant genes Vrn. - Czech J. Genet. Plant Breed. **37**: 41-49, 2001b.

Limin, A.E., Fowler, D.B.: Developmental traits affecting low-temperature tolerance response in near-isogenic lines for the vernalization locus *Vrn-A1* in wheat (*Triticum aestivum* L. em Thell). - Ann. Bot. **89**: 579-585, 2002.

Mahfoozi, S., Limin, A.E., Fowler, D.B.: Influence of vernalization and photoperiod responses on cold hardiness in winter cereals. - Crop Sci. **41**: 1006-1011, 2001a.

Mahfoozi, S., Limin, A.E., Fowler, D.B.: Developmental regulation of low-temperature tolerance in winter wheat. - Ann. Bot. **87**: 751-757, 2001b.

Petr, J., Hnilička, F.: Changes in requirements on vernalization of winter wheat varieties in the Czech Republic in 1950-2000. - Rost. Výroba **48**: 148-153, 2002.

Prášil, I., Prášilová, P., Pánková, K. Relationships among vernalization, shoot apex development and frost tolerance in wheat. - Ann. Bot. **94**: 413-418, 2004.

Prášil, I., Prášilová, P., Papazisis, K., Valter, J.: Evaluation of freezing injury and dynamics of freezing resistance in cereals. - In: Dörffling, K., Brettchneider, B., Tantau, H., Pithan, K. (ed.): Crop Adaptation to Cool Climates. Pp. 37-48. ECSP-EEC-EAEC, Brussels 1994.

Prášil, I., Rogalewicz, V.: Accuracy of wheat winterhardiness evaluation by a provocation method in natural conditions. - Genet. Šlecht. **25**, 223-230, 1989.

Roberts, D.W.A.: Duration of hardening and cold hardiness in winter wheat. - Can. J. Bot. **57**: 1511-1517, 1979.

Roberts, D.W.A.: 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. 1990.

Roberts, D.W.A., MacDonald, M.D.: Evidence for the multiplicity of alleles at *Vrn1*, the winter-spring habit locus in common wheat. - Can. J. Genet. Cytol. **26**: 191-193, 1984.

Roberts, D.W.A., MacDonald, M.D.: Role of chromosome 5A in wheat in control of some traits associated with cold hardiness of winter wheat. - Can. J. Bot. **66**: 658-662, 1988.

Schmütz, W.: Neuere Ergebnisse zur Beziehung zwischen Vernalisation und Winterfestigkeit bei Getreide. - In: Bericht über die Arbeitstagung der Arbeitsgemeinschaft der Saatzuchtleiter. Pp. 77-86. Gumpenstein 1977.

Snape, J.W., Sarma, R., Quarrie, S.A., Fish, L., Galiba, G., Sutka, J.: Mapping genes for flowering time and frost tolerance in cereals using precise genetic stocks. - Euphytica **120**: 309-315, 2001.

Sutka, J.: Genes for frost resistance in wheat. - Euphytica **119**: 167-172, 2001.

Sutka, J., Kovács, G.: Reciprocal monosomic analysis of frost resistance on chromosome 5A in wheat. - Euphytica **34**: 367-370, 1985.

Sutka, J., Kovács, G., Veisz, O.: Substitution analysis of the frost resistance and winter hardiness of wheat under natural and artificial conditions. - Cereal Res. Commun. **14**: 49-53, 1986.

Tóth, B., Galiba, G., Fehér, E., Sutka, J., Snape, J.W.: Mapping genes affecting flowering time and frost on chromosome 5B of wheat. - Theor. appl. Genet. **107**: 509-514, 2003.

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

Vágújfalvi, A., Galiba, G., Cattivelli, L. Dubcovsky, J.: The cold-regulated transcriptional activator *Cbf3* is linked to the frost-tolerance locus *Fr-A2* on wheat chromosome 5A. - Mol. gen. Genet. **269**: 60-67, 2003.

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