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ARTICLE

Effect of chromosome 5A on gene expression during cold hardening in wheat

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ABSTRACT Changes in the transcript profile during cold hardening, a treatment necessary for achieving the full level of freezing tolerance, were monitored in a genetic system of wheat (*Triticum aestivum* L.) chromosome 5A substitution lines differing in their freezing tolerance. The number of cold-responsive genes in a freezing-tolerant substitution line (146) was significantly higher than in a sensitive one (97), indicating a general relationship between the overall number of genes with altered expression and the degree of freezing tolerance. The expression of 175 genes was differentially affected in the freezing-tolerant and sensitive substitution lines. Three of them, coding for a Ca-binding protein (Cab), a cold-responsive protein (Tacr7) and a protein described in a mutant deficient in embryo and meristems (Dem), were characterised in more detail. The expression of Dem was induced only by low temperature, while the transcript level of Cab was also higher following NaCl treatment and that of Tacr7 after salicylic acid and H_2O_2 application.
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KEY WORDS

cold acclimation
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Winter frosts can cause severe damage and consequently yield loss in winter cereals, so tolerance to freezing temperatures is a very important agronomic trait. Cold hardening which occurs during autumn under natural conditions is essential if the genetically determined level of freezing tolerance is to be achieved. During cold acclimation coordinated changes take place in plants at the gene expression level. Several genes involved in the response to low temperature and other stresses were localised on the long arm of chromosome 5A in wheat, which has a major effect on freezing tolerance (Sutka 1994). Two loci, *Fr-A1* and *Fr-A2*, controlling freezing tolerance were mapped on the long arm of chromosome 5A (Galiba et al. 1995; Vágújfalvi et al. 2003). In addition, it was shown that *CBF* transcription factors regulating the expression of several cold-responsive genes are linked to the *Fr-A2* locus (Vágújfalvi et al. 2003; 2005). Besides *Fr-A1* and *Fr-A2*, two vernalisation genes, *Vrn-A1* and *Vrn-A2*, were also localised on chromosome 5A (Galiba et al. 1995; Danyluk et al. 2003). These genes are responsible for the fact that winter wheats require a long period of low temperature if they are to be capable of flowering. The *Vrn* genes act as a master switch controlling the expression length of low temperature-induced structural genes (Danyluk et al. 2003).

The aim of this study was to compare a set of cold-responsive genes and to identify genes regulated by chromosome 5A and responsible for freezing tolerance in wheat by carrying out transcript profiling in chromosome 5A substitution lines with different levels of freezing tolerance.

Materials and Methods

Cold-induced changes in the transcript profile were compared in a specific genetic system consisting of the moderately freezing-sensitive (spring habit) recipient wheat variety Chinese Spring [*Triticum aestivum ssp. aestivum,* CS], as well as the freezing-sensitive (spring habit) CS(*T. a. ssp. spelta* 5A) [CS(Tsp5A)] and the freezing-tolerant (winter habit) CS(*T. a. ssp. aestivum* cv. Cheyenne 5A) [CS(Ch5A)] chromosome 5A substitution lines (Vágújfalvi et al. 1999). The plants were cultivated as described earlier (Kocsy et al. 2000). The following treatments were used: 2°C 21 d, 1 mM H_2O_2 7 d, 0.5 mM salicylic acid 7 d and 200 mM NaCl 7 d.

The cDNA-macroarray (10297 unique EST sequences) analysis was done according to Zierold et al. (2005) except for the less stringent washing of the wheat samples after hybridization (1 x SSC, 0.1% SDS; 0.5 x SSC, 0.1% SDS) due to the heterologous nature of the hybridization. Expression changes in the case of genes Cab, Dem and Tacr7 were confirmed by Northern analysis (Vallelian et al. 1998) and real time RT-PCR (Altpeter et al. 2005) as described previously. Semiquantitative RT-PCR (Kellős et al. 2008) was used to test the effect of different treatments on the expression of the selected genes. Data analysis was done as described previously (Zierold et al. 2005).

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Table 1. Number of cold-responsive genes in the freezing sensitive Chinese Spring (CS) variety and CS(Cheyenne 5A) [CS(Ch5A)] chromosome substitution line and in the freezing-tolerant CS(Triticum spelta 5A) [CS(Tsp5A)] line.

	CS	CS(Tsp5A)	CS(Ch5A)
Total	365	322	428
Without overlap	134	97	146

Results and Discussion

In contrast to freezing tolerance, which exhibited a maximum after 3 weeks of hardening (Vágújfalvi et al. 1999), the greatest absolute and relative changes in gene expression occurred during the first week of hardening. These early changes in the transcript profile may have initiated such specific changes at the proteome and metabolome levels in the recipient CS and in the two 5A chromosome substitution lines which resulted in the phenotypic differences in freezing tolerance being maximum after 21 d hardening. A larger number of cold-responsive genes were detected in the freezing-tolerant line CS(Ch5A) than in the other genotypes, suggesting a general relationship between the overall number of genes with altered expression and the degree of freezing tolerance (Table 1).

Regarding the three genes selected for detailed analysis on the basis of significant differences in their cold-induced expression changes in freezing-tolerant and sensitive genotypes, the continuous increase in the *Tacr7* transcript level indicates that it plays a role throughout the cold hardening process. Similarly to the present results, Gana, Sutton and Kenefick (1997) found higher *Tacr7* expression in a freezing-tolerant wheat genotype compared to a sensitive one. The amount of *Tacr7* transcript was also higher after salicylic acid and H_2O_2 application. Sequence comparisons demonstrated that the other interesting candidate, the *Cab* gene, differed from other Ca^{2+}-binding proteins induced by low temperature (Fowler and Thomashow 2002) or other abiotic stresses (Seki et al. 2001). Since Ca^{2+} is involved in cold signalling (Scrase-Field and Knight 2003), the Cab protein may have a regulatory role during cold acclimation. This assumption is corroborated by the rapid transient induction of *Cab*, which was greater in the freezing-tolerant genotype than in the sensitive ones. *Cab* expression was also induced by NaCl treatment. The cold induction of the 3rd candidate gene, *Dem*, was not reported earlier. Its expression was greater in CS(Ch5A), a vernalization-sensitive substitution line, than in the vernalization-insensitive CS(Tsp5A) line and the recipient CS (Galiba et al. 1995). Since *Dem* plays an important role in the apical meristems (Keddie et al. 1998) it might be part of the Vrn-regulon.

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References

Altpeter F, Varshney A, Abderhalden O, Douchkov D, Sautter C, Kumlehn J, Dudler R, Schweizer P (2005) Stable expression of a defence-related gene in wheat epidermis under transcriptional control of a novel promoter confers pathogen resistance. Plant Mol Biol 57:271-283.

Danyluk J, Ndijido AK, Breton G, Limin AE, Fowler BD, Sarhan F (2003) *TaVRT-1*, a putative transcription factor associated with vegetative to reproductive transition in cereals. Plant Physiol 132:1849-1860.

Fowler S, Thomashow MF (2002) *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell 14:1675-1690.

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

Gana JA, Sutton F, Kenefick DG (1997) cDNA structure and expression pattern of a low-temperature-specific wheat gene *tacr7*. Plant Mol Biol 34:643-650.

Keddie JS, Carroll BJ, Thomas CM, Reyes MEC, Klimyuk V, Holtan H, Gruissem W, Jones JDG (1998) Transposon tagging of the *Defective embryo and meristems* gene of tobacco. Plant Cell 10:877-887.

Kellős T, Tímár I, Szilágyi V, Szalai G, Galiba G, Kocsy G (2008) Stress hormones and abiotic stresses have different effects on antioxidants in maize lines with different sensitivity. Plant Biol doi:10.1111/j.1438-8677.2008.00071.x

Kocsy G, Szalai G, Vágújfalvi A, Stéhli L, Orosz G, Galiba G (2000) Genetic study of glutathione accumulation during cold hardening in wheat. Planta 210:295-301.

Seki M, Narusaka M, Abe H, Kasuga M, Yamaguochi-Shinozaki K, Carninci P, Hayashizaki Y, Hshinozaki K (2001) Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full length cDNA microarray. Plant Cell 13:61-72.

Sutka J (1994) Genetic control of frost resistance in wheat (*Triticum aestivum* L.). Euphytica 77:77-282.

Vágújfalvi A, Aprile A, Miller A, Dubcovsky J, Delugu G, Galiba G, Cattivelli L (2005) The expression of several Cbf genes at the Fr-A2 locus linked to frost resistance in wheat. Mol Genet Genom 274:506-514.

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

Vágújfalvi A, Kerepesi I, Galiba G, Tischner T, Sutka J (1999) Frost hardiness depending on carbohydrate changes during cold acclimation in wheat. Plant Sci 144:85-92.

Vallelian BL, Mosinger E, Metraux JP, Schweizer P (1998) Structure, expression and localisation of a germin-like protein in barley *Hordeum vulgare* L. that is insolubilised in stressed leaves. Plant Mol Biol 37:297-308.

Zierold U, Scholz U, Schweizer P (2005) Transcriptome analysis of mlo-mediated resistance in the epidermis of barley. Mol Plant Pathol 6:139-151.