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Comparison of the antioxidant capacity in cold-treated recombinant wheat lines

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ABSTRACT In earlier experiments using 5A chromosome substitution lines of wheat with different freezing tolerance it was shown that this chromosome plays an important role in the regulation of cold-induced changes in the level of antioxidant enzymes and thiols. The purpose of our experiments was to determine the region of chromosome 5A where the genes responsible for the control of stress-induced changes in the level of the antioxidants are localised. Activities of antioxidant enzymes and the glutathione synthesis were compared in lines recombinant for the long arm of chromosome 5A. The chromosome region between the markers Xpsr805 and Xpsr164 was found to influence the cold-induced changes in hydroxymethylglutathione and glutathione levels. The glutathione reductase activity may be affected by the chromosome region near to the marker B-amy-A1. **Acta Biol Szeged 49(1-2):117-119 (2005)**

KEY WORDS

antioxidant
cold
chromosome 5A
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The different abiotic environmental stresses, similarly to biotic stresses, induce oxidative stress in living beings because of the accumulation of reactive oxygen species (ROS). The increase in the concentration of ROS initiates such signal transduction processes which activate protective mechanisms, involving the increase in the level of the antioxidants. The antioxidants protect against molecular lesions which can result in yield loss due to the inhibition of plant growth and development.

Catalase and the ascorbate-glutahione cycle participates in the removal of hydrogen peroxide (Noctor et al. 1988) wich may be accumulated during low-temperature-induced oxidative stress (O'Kane et al. 1996). An important component of the ascorbate-glutathione cycle, glutathione (GSH) is synthesized in two steps (Brunold and Rennenberg 1997): first cysteine and glutamate are bound to γ-glutamycysteine (γEC) by γEC synthetase, then a glycine is added to the dipeptide by GSH synthetase. In Gramineae a homologue of GSH, hydroxymethylglutathione (hmGSH) is also present, in which glycine is replaced by serine (Klapheck et al. 1991). Besides its role in the H_2O_2 removal, GSH participates in the detoxification of lipid hydroperoxides which reaction is catalased by glutathione S-transferase (GST).

The effect of cold hardening on the accumulation of GSH, hmGSH and its precursors and on glutathione reductase (GR) activity was studied in wheat (Kocsy et al. 2000). In these experiments it was shown that GSH and hmGSH accumulation contributed to enhanced frost tolerance in wheat, and the chromosome 5A participated in the regulation of thiol levels and their redox state and GR activity. Changes in the activities of antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT] and peroxidases) induced by cold acclimation support the hypothesis that a frost-resistant wheat cultivar, in comparison with a less frost-resistant one, maintains a better defence against ROS during low-temperature treatment (Scebba et al. 1998).

Materials and Methods

The frost-tolerant chromosome 5A substitution line Chinese Spring (Cheyenne 5A) [CS/CNN 5A] and the frost-sensitive line Chinese Spring (T. spelta 5A) [CS/TSP 5A] and the single chromosome (for 5A) recombinant lines 7/3, 32/5, 34/3, 4/4, 56/1, 12/4 (developed by the hybridization of the two substitution lines) were used in the experiments (Fig. 1). The seeds were obteined from the Martonvásár Cereal Gene Bank (Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary).

After germination in Petri dishes (25°C, 3d) the seedlings were cultivated in hydroponics in a spring type growth chamber (Conviron PGV-36 chamber, Controlled Env. Ltd., Winnipeg, Canada) at 18/15°C day/night temperature and 70/75% relative humidity, with 16 h illumination at 260 μmol m^{-2} s^{-1} for 10 d. The subsequent cold treatment lasted for 7 d at 2°C.

The reduced and oxidised thiols were determined as described by Kocsy et al. (2005), after separation by reverse-phase HPLC (Waters) using fluorescense (W 474 scanning fluorescense detector; Waters) detection.

The CAT activity was determined as Aebi (1983) and the ascorbate peroxidase [APX], GR and glutathione-S-transferase [GST] activities and protein content were measured according to Kocsy et al. (2005) using a Cary-100 UV-Vis spectrophotometer (Varian, Middelburg, The Netherlands).

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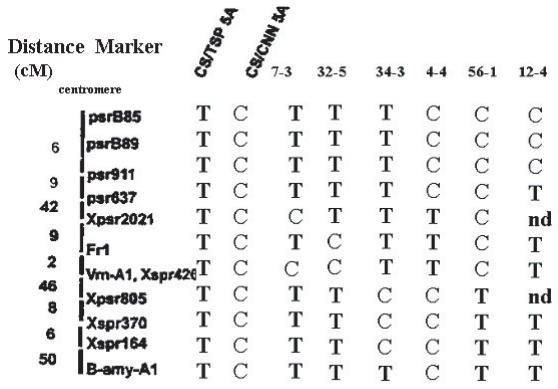

Figure 1. The origin of the chromosome regions on long arm of chromosome 5A in the recombinant lines. The RFLP probes representing TSP and CNN alleles are indiced by T and C, respectively; nd, allele not determined (Vágújfalvi et al. 2000).

Results

Cold treatment induced different changes in the GSH and hmGSH synthesis of the two parental lines, since the decrease in the hmGSH level was greater in CS(TSP5A) and the increase in GSH content was greater in CS(CNN5A; Figs. 2 and 3). Similarly, there was also a different alteration in cysteine levels in the two substitution lines (data not shown). The GSH and hmGSH levels in the recombinant lines 34/3 and 4/4 showed similar changes during the experiment as observed in the CS(CNN5A) substitution line. Although the oxidised form of all four thiols was lower in the substitution lines following cold treatment, it increased in some recombinant lines during the experiment (data not shown). There was a significant difference between the substitution lines in the cold-induced changes of cystine and oxidised hydroxymethylglutathione (hmGSSG) content. The ratio of reduced to oxidised thiols increased in both parental lines during the cold treratment (data not shown).

The activity of APX was not affected in the two substitution lines by cultivation at 2°C, however its significant change was observed in some recombinant lines (data not shown). Following cold teratment the activity of GR transiently decreased in both parental lines then increased, but this increase was greter in CS(CNN 5A) than in CS(TSP5A) (data not shown). Growth at 2°C resulted in similar changes of GR activity in the recombinant line 34/3 as described for (CS(CNN5A). The GST activity did not change following cold treatment in CS(CNN5A) while it decreased in CS(TSP5A) (data not shown). In the recombinant lines only a transient decrease in GST activity was found. The Cat activity was higher after 3 d at 2°C, but later on decreased to the starting level in CS(CNN5A) (data not shown). It was not affected by cold treatment in the other substitution line and in the most recombinant lines.

Discussion

Cold treatment, similarly to previous findings (Kocsy et al. 2000), induced a greater increase in the GSH content in the frost-tolerant CS(CNN5A) compared to the frost-sensitive CS(TSP5A). The similar cold-induced changes in hmGSH and GSH levels in CS(CNN5A) and the recombinant lines 34/3 and 4/4 indicate that the chromosome region between the markers Xpsr805 and Xpsr164 affects the cocentrations of these thiols at low temperature (Fig. 1).

An affect of chromosome 5A on cold-induced changes in the GR, GST and Cat activity was demonstrated in the present study which corrobarates the previous findings in case of GR (Kocsy et al. 2000). The results obtained using the recombinant lines show that GR activity may be affected by

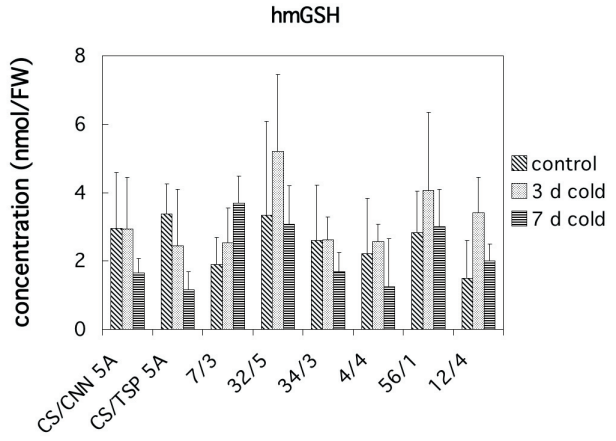

Figure 2. Effect of cold treatment on reduced hydroxymethylglutathione (hmGSH) content in wheat. Values indicated with different letters are significantly different at the p<5% level.

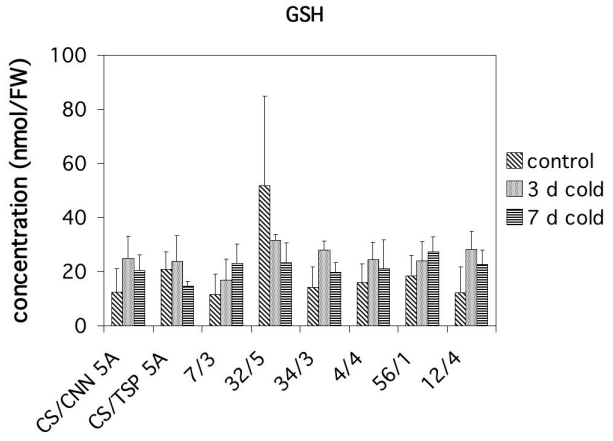

Figure 3. Effect of cold treatment on reduced glutathione (GSH) content in wheat. Values indicated with different letters are significantly different at the p<5% level.

the chromosome region near to the marker B-amy-A1. Baek and Skinner (2003) could show that a neighbouring region of chromosme 5A in wheat controls the cold-induced expression of the gene coding for catalase.

Taken together, the regions of chromosome 5A affecting the cold-induced changes in the GSH and hmGSH levels and GR activity were identified. To narrow these chromosome regions additional experiments are necessary with the involvement of more recombinant lines in the future.

Acknowledgments

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