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ARTICLE

Regulation of free amino acid and polyamine levels during cold acclimation in wheat

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ABSTRACT The effect of cold acclimation on free amino acids and polyamines was compared at metabolite and transcript level in wheat chromosome 5A substitution lines with different freezing tolerance. Three weeks at 2°C resulted in increased H_2O_2 content which alteration may affect the metabolism of these compounds. The concentration of most free amino acids gradually increased during the treatment. The expression of the genes encoding enzymes of amino acid metabolism, thus that of pyrroline-5-carboxylate reductase, glutamate synthetase and aspartate transferase had a fast transient increase during the first days of growth at 2°C. The concentration of the polyamines putrescine and spermidine exhibited a great increase in all three genotypes, while spermine and cadaverine levels showed only slight changes. Among the genes related to the polyamine metabolism, the transcript level of those ones encoding arginine and ornithine decarboxylase increased and that of the spermidine synthetase did not change. The observed changes in metabolite and transcript levels were controlled by chromosome 5A in the case of Pro, Glu and putrescine. The present result show that free amino acids and polyamines play an important role in the cold acclimation and their levels are regulated at the transcriptional level

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KEY WORDS

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Cold acclimation is necessary for winter wheat (*Triticum aestivum* L.) varieties to achieve the genetically determined maximum freezing tolerance. Chromosome 5A is a major regulator of this trait. Free amino acids and polyamines take part in several metabolic processes and they are involved in the protection against abiotic stresses. The amino acids have several roles in plants, for example they act as osmolytes, detoxify heavy metals, regulate ion transport, stomatal opening, affect synthesis and activity of enzymes, gene expression and redox homeostasis (Rai 2002). Positively charged polyamines are involved in the stress response through their interaction with the negatively charged macromolecules, such as DNA, RNA, proteins and phospholipids, resulting changes in the physical and chemical properties of the membranes, in the structure of nucleic acids and in the enzyme activities (Alcázar et al. 2006). In addition, polyamines are able to detoxify the reactive oxygen species accumulating during abiotic stress.

The aim of the present study was to find out whether cold acclimation affects free amino acids and polyamines at gene expression and metabolite level and this effect is regulated by chromosome 5A in wheat.

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Materials and Methods

Seedlings of the moderately freezing-sensitive wheat (*Triticum aestivum* ssp. *aestivum*) variety Chinese Spring (CS), and the freezing-sensitive CS(*T. ae*. ssp. *spelta* 5A) [CS(Tsp5A)] and freezing-tolerant CS(*T. ae.* ssp. *aestivum cv.* Cheyenne 5A) [CS(Ch5A)] chromosome substitution lines were grown in hydroponics. After two weeks the plants were subjected to no treatment, were cultivated in the presence of 1 mM H_2O_2 for 1 day, at 19°C or 14°C for 1 day, or were cold-acclimated at 2°C for 0, 1, 3, 7 and 22 days.

The transcript level of the genes encoding enzymes of amino acid and polyamine metabolism was measured by oligonucleotide microarray and by semiquantitative RT-PCR (Kovács et al. 2010). The concentration of free amino acids and polyamines was determined by amino acid analyzer (Kovács et al. 2010, 2011). All biochemical parameters were measured in the shoots.

Results and Discussion

According to the microarray analysis the expression of certain genes encoding enzymes of amino acid metabolism was changed even by a slight reduction in the temperature and some of them was affected by H_2O_2 (Fig. 1). From the semiquantitative RT-PCR analysis turned out that the

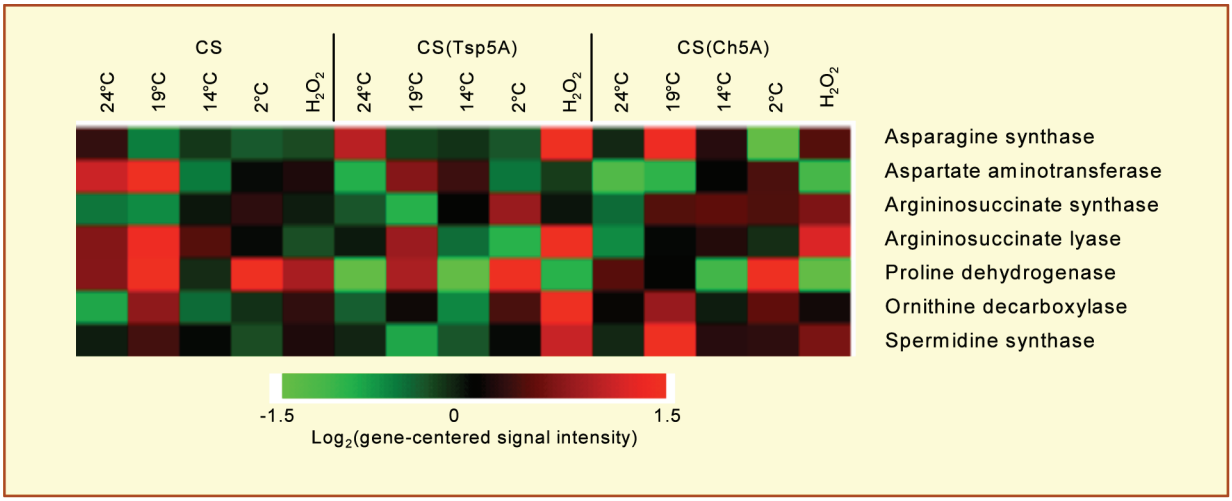

Figure 1. Effect of various suboptimal temperatures and H_2O_2 on the expression of the genes encoding enzymes of amino acid and polyamine metabolism.

expression of the genes encoding enzymes of Asp, Glu and Pro metabolism exhibited a rapid transient initial increase with a maximum after 3 days at 2°C which was followed by a gradual decrease. The Asn, Gln and Pro content increased throughout the 3 weeks cold treatment, while the Asp and Glu concentration showed an increase only during the first 3 days in the freezing-sensitive CS and CS(Ch5A) and during the first week in the freezing-tolerant CS(Tsp5A).

The increase in Glu concentration during cold acclimation is very important in order to improve the freezing tolerance of wheat since Glu is a precursor not only of Pro, but also of glutathione (GSH) and polyamines which molecules all are involved in the protection against the adverse environmental effects. Thus, the co-ordinated use of Glu for Pro, GSH and polyamine synthesis should exist under stress conditions which ensure the necessary amount of these metabolites. Similarly to the present study, the cold-induced accumulation of Pro in wheat was also observed by Macháčková et al. (2006). The importance of Pro in the cold acclimation process was shown by the much greater increase (1.5- and 2-fold) of its concentration in the freezing-tolerant CS(Ch5A) genotype compared to the freezing-sensitive CS and CS(Tsp5A).

From the genes encoding enzymes of polyamine metabolism, the expression of ornithine decarboxylase and spermidine synthase was affected by both a moderate decrease in temperature and H_2O_2 as it was shown by the microarray analysis (Fig. 1). By semiquantitative RT-PCR a great elevation was detected in the transcript level of the gene encoding ornithine decarboxylase during the first week of cultivation at 2°C. The increase in the expression was transient in the case of the gene of spermidine synthase, while the transcript of Arg decarboxylase exhibited only smaller changes. The putrescine content increased during the 3 weeks of cold treatment with a transient decrease during the first week. The concentration of spermidine and spermine also exhibited a transient decrease at the beginning of the cultivation at 2°C and later on only their smaller changes were observed.

Similarly to the present study, the cold-induced alterations in Put content depended on the level of freezing tolerance, when other wheat genotypes with different levels of freezing tolerance were compared (Szalai et al. 2009). In *Arabidopsis* Put was suggested to affect freezing tolerance and cold acclimation through the control of ABA levels (Cueves et al. 2008). The regulatory role of polyamines was also demonstrated in transgenic *Arabidopsis* overexpressing the gene encoding the ADC enzyme, which resulted in increased Put content and the down-regulation of several genes encoding transcription factors involved in the response to abiotic stress (Alcázar et al. 2005).

It can be concluded that the great increase in Asp, Glu and putrescine contents during the first week of cold treatment may be a result of the increased transcription of the corresponding genes. According to the changes in its transcript level ornithine decarboxylase may have a dominant role in the putrescine synthesis at low temperature. The cold-induced changes of amino acids and polyamines at transcript and metabolite levels correlated with the level of freezing tolerance and were affected by chromosome 5A only in the case of Pro, Glu and putrescine.

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