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ARTICLE

Effect of cold treatment on biogenic amine content in wheat

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ABSTRACT The effect of cold treatment on concentration of biogenic amines was compared in the moderately frost-sensitive *Triticum aestivum* cv. Chinese Spring wheat genotype and in the frost-tolerant Chinese Spring (Cheyenne 5A) and the frost-sensitive Chinese Spring (*T. spelta* 5A) chromosome 5A substitution lines. To see the dynamics of changes, the plant material was collected after 0d, 1d, 3d, 7d and 21d of treatment at 2°C. Quantitative determination of biogenic amines was accomplished by chromatographic method. The cold treatment caused especially great increase in putrescine and spermidine levels. Chromosome 5A affected the cold induced increase in putrescine content, being larger after 21d of cold treatment in the frost-tolerant Chinese Spring (Cheyenne 5A) compared to the sensitive genotypes, which may indicate the role of putrescine accumulation in frost tolerance. **Acta Biol Szeged 52(1):195-197 (2008)**

KEY WORDS

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Low temperature, one of the most important environmental stresses, can adversely affect the growth, development and agricultural production of plants. It induces many alterations in biochemistry and physiology of plants, for example in protein and membrane composition, in the activities of enzymes and ion channels and accumulation of sugars, free amino acids and other compounds such as biogenic amines.

Biogenic amines are low molecular weight organic bases that possess biological activity. They can be formed and degraded as a result of normal metabolic activity in plants, animals and microorganisms, and are usually produced by the decarboxylation of amino acids (Halász et al. 1994). In plants, polyamines (spermidine, spermine and their precursor putrescine) are the most common biologically active amines. Polyamines are implicated in a number of physiological processes, such as promotion of growth, cell division, DNA replication and cell differentiation (Groppa and Benavides 2008). Numerous studies support the view that polyamines not only involved in fundamental cellular processes, but also play essential roles in abiotic stress tolerance. During stress, polyamines act as reactive oxygen species scavenging, protect membranes and macromolecules and play a role as signalling molecules in expression of several stress-related genes (Bouchereau et al. 1999; Alcázar et al. 2006; Groppa and Benavides 2008).

The aim of this work was to investigate whether cold treatment and/or chromosome 5A of wheat, a main regulator of cold tolerance, influence the level of biogenic amines.

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Materials and Methods

Plant material. The moderately frost-sensitive *Triticum aestivum* cv. Chinese Spring [CS] wheat cultivar and the frost-tolerant Chinese Spring (Cheyenne 5A) [CS(Ch5A)] and the frost-sensitive Chinese Spring (*T. spelta* 5A) [CS(Tsp5A)] chromosome 5A substitution lines were used in experiments. The seeds were obtained from the Martonvásár Cereal Gene Bank (Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary).

Growth conditions. The seeds were germinated between wet filter papers at 25°C for 1 day, then at 4°C for 2 d to synchronise the germination and during the last 2 d they were kept at 25°C again. The plants were grown in pots containing half-strength Hoagland nutrient solution (Hoagland and Arnon 1950) in an autumn-winter type growth chamber (Conviron PGV-36, Controlled Environ. Ltd., Winnipeg, Canada) for 12 d at 18/15°C day/night temperature and 70/75% relative humidity, with 16 h illumination at 270 μmol $m^{-2}s^{-1}$. The cold treatment lasted at 2°C for 21 d, the other growth parameters were not changed. The plant material for the biochemical analysis was collected at the beginning of cold hardening and after 1d, 3d, 7d and 21d of treatment.

Sample preparation for biogenic amine determination. Samples (300-600 mg FW) crushed in liquid nitrogen were extracted with 2 mL cold 10% trichloroacetic acid for 1 hour with gentle agitation at room temperature on a shaker (Gerhardt GmbH & Co., Germany). The supernatant was collected after centrifugation at 5000 rpm for 10 min. Each supernatant was filtered through a 0,2 μm pore membrane filter (Sartorius AG, Germany). The analysis of the extract was carried out

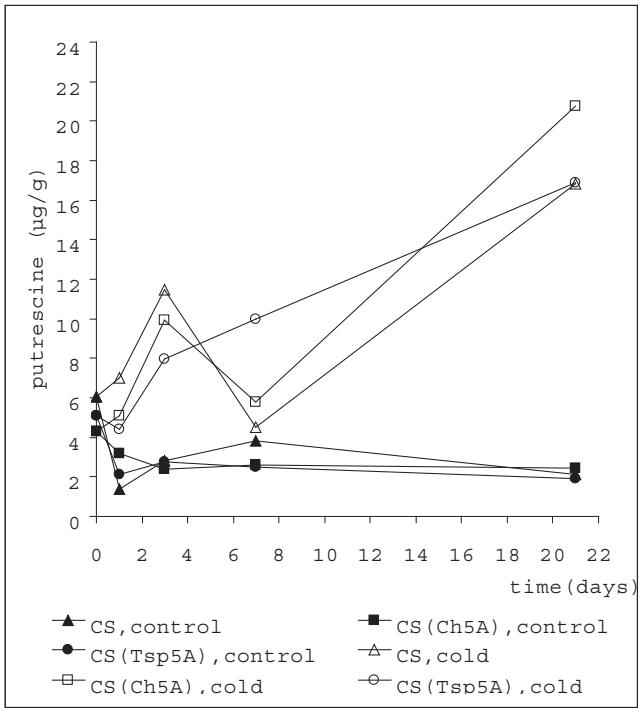

Figure 1. Changes of putrescine content in wheat genotypes during cold treatment.

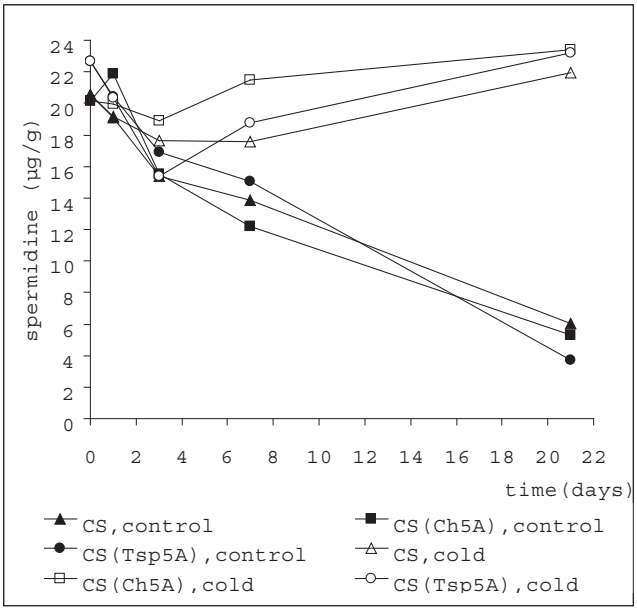

Figure 2. Changes of spermidine content in wheat genotypes during cold treatment.

on an automatic amino acid analyzer (Ingos AAA400, Czech Republic). The biogenic amines were detected with ninhydrin at 570 nm.

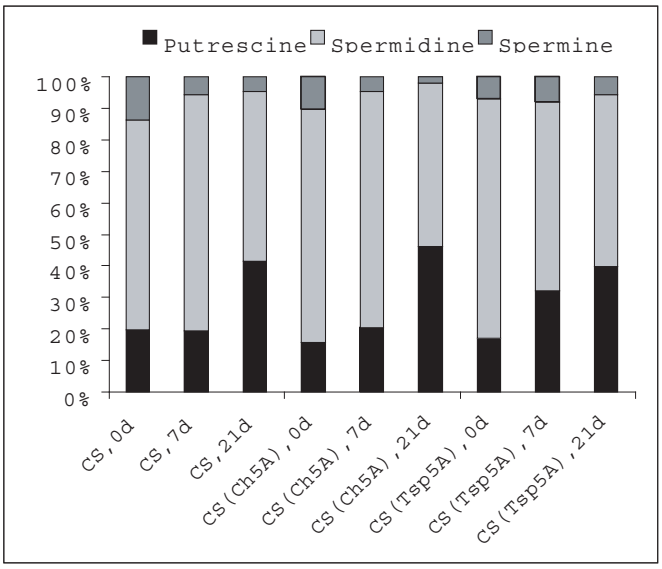

Figure 3. Percentage of putrescine, spermidine and spermin during cold treatment.

Results and Discussion

Seven biogenic amines were investigated in the experiments. Putrescine, spermidine and spermine were present in all samples, while agmatine and cadaverine were detectable neither in the control nor in the cold-treated plants. Histamine and tyramine were shown only in a part of samples at low levels (0.38-4.96 µg/g).

The cold treatment resulted in a great increase in the putrescine level in all genotypes after 3d (Fig. 1). Approximately 4-fold being observed in CS and CS(Ch5A) and 3-fold in CS(Tsp5A). After 21d, the amount of putrescine was 8-9 times higher in the cold-treated samples than in the counterpart control varieties. The frost-tolerant CS(Ch5A) had 23% higher level of putrescine compared with the frost-sensitive genotypes. After 7d, a significant decrease was observed in putrescine level of cold-treated CS and CS(Ch5A). Probably the utilization of putrescine was greater during this time than the cold induced accumulation.

Contrary to putrescine, cold hardening did not change the spermidine content. The great difference between treated and control plants was due to the large decrease in spermidine content in control plants. After 7d of treatment, there was 1.3-fold, 1.8-fold and 1.2-fold difference in spermidine level between the cold-hardened CS, CS(Ch5A) and CS(Tsp5A) genotypes and the equivalent control plants, respectively (Fig. 2). After 21d, the difference was much higher (3.6-fold, 4.4-fold and 6.3-fold, respectively).

The percentage of polyamines is shown at Figure 3. Rate of putrescine represents 20% and 15% of total polyamine content in the beginning of the cold treatment in CS and CS(Ch5A) genotypes. The putrescine ratio showed only slight

increase during short term cold hardening in these genotypes. However, after 21d, the putrescine ratio became doubled at the expense of spermidine and spermine. In CS(Tsp5A), the ratio of putrescine increased approximately two times by the end of the long term cold hardening like in CS and CS(Ch5A), but the dynamics of putrescine accumulation were different from those genotypes. In this chromosome substitution line, the putrescine ratio increased gradually parallel to the decrease of spermidine.

The results indicate that cold-induced changes in the putrescine content are affected by the chromosome 5A and play an important role in the response to low temperature stress in wheat. Probably spermidine and spermine are involved in the defence against the cold stress by their alterations into putrescine.

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