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Effect of heat stress on glutathione biosynthesis in wheat

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ABSTRACT A relationship was found between the frost sensitivity and glutathione accumulation during cold treatment in wheat. The aim of the present study was to investigate in the same genotypes whether there is also a relationship between the frost sensitivity and the glutathione accumulation during high temperature stress. The glutathione and hyroxymethylglutathione content as well as the activity of the two enzymes of glutathione synthesis was greater in the frost-sensitive genotypes than in the tolerant ones during heat stress. High temperature stress resulted in a greater ratio of the reduced to oxidised non-protein thiols and the greater glutathione reductase activity in the sensitive genotypes. Thus, it can be established that the GSH accumulation induced by heat stress depends on the frost sensitivity of wheat.
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KEY WORDS

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Glutathione (GSH), as an important component of the ascorbate-glutathione cycle, participates in the removal of hydrogen peroxide (for reviews see Noctor and Foyer 1998) which may be accumulated during high temperature-induced oxidative stress. GSH is synthesized in two steps: first cysteine and glutamate are bound to γ-glutamylcysteine (γEC) by γEC synthetase, then a glycine is added to the dipeptide by GSH synthetase. During oxidative stress GSH will be oxidised and it is regenerated by glutathione reductase (GR). In *Gramineae* a homologue of GSH, hydroxymethylglutathione (hmGSH), is also present, in which glycine is replaced by serine (Klapheck et al. 1991).

Nieto-Sotelo (1989) found an increase of GSH content and a decrease of cysteine content in maize subjected to heat stress which changes were due to a greater rate of GSH synthesis. In maize GSH increased only in the tolerant genotype after cultivation at 40°C for 3 d (Kocsy et al. 1998a). Heat stress increased the amount of hmGSH in wheat (Kocsy et al. 1998b). From these results can be established that GSH synthesis is induced not only by low, but also by high temperature. The aim of the present study was to compare the effect of heat stress on GSH synthesis in wheat genotypes with different frost sensitivity.

Materials and Methods

The frost-tolerant *Triticum aestivum* L. cv. Cheyenne, the moderately sensitive *T. aestivum* cv. Chinese Spring, a frost-sensitive *T. spelta* L. accession and the chromosome substitution lines Chinese Spring (Cheyenne 5A) and Chinese Spring (*T. spelta* 5A) were used in the experiments. The seeds were germinated between wet filter papers at 25 °C. The plants were grown on Hoagland-solution in an autumn-winter type growth chamber at 15/10 °C day/night temperature for 2 weeks with 16 h illumination at 260 mmol m^{-2} s^{-1}, then the heat treatment was carried out at 37 °C for 3 d. The reduced and oxidised non-protein thiols were determined by HPLC using fluorescence detection and the glutathione reductase activity was measured by spectrophotometer as described previously (Kocsy et al. 2000). The in vivo glutathione synthesis was followed using [^{35}S]sulfate and the radioactivity incorporated into the thiols was determined by HPLC using radioactive detection (Kocsy et al. 2000).

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Results and Discussion

After 3 d heat stress the highest reduced cysteine, hmGSH and GSH levels and GR activity were found in the frost sensitive Tsp and CS(Tsp5A). The amount of the oxidative form of these thiols reached the highest level in the frost-tolerant Ch and CS(Ch5A) genotypes. The ratio of reduced to oxidised cysteine, GSH and hmGSH was the greatest in the frost-sensitive Tsp and CS(Tsp5A). The activity of γEC and glutathione synthetase increased after heat stress in all genotypes examined, and their highest levels were found in the two sensitive ones. The greatest induction of cysteine and hmGSH synthesis was observed in Tsp and CS(Tsp5A). The amount of radioactive GSH decreased after heat stress in all genotypes. The smallest decrease occurred in Tsp and CS(Tsp5A).

A relationship between the frost sensitivity and GSH synthe-sis at high temperature was found in wheat, since the highest reduced GSH and hmGSH levels, reduced to oxidised thiol ratios, GR activity, in vivo GSH and hmGSH synthesis were detected in the frost-sensitive Tsp and CS(Tsp5A) during heat stress. This results also shows, that like during low temperature stress (Kocsy et al. 2000), during high tempera-ture stress the 5A chromosome of wheat also affects GSH and hmGSH accumulation. Contrary to high temperatury stress, low temperature stress caused the highest increase in GSH and hmGSH contents in the frost-tolerant Ch and CS(Ch5A). Thus, GSH and hmGSH had a role both in the tolerance to extreme low and high temperatures. As observed in wheat the amount of oxidised glutathione also increased in durum wheat and mustard (Paolacci et al. 1997; Dat et al. 1998). Contrary with the present results, the GSH content increased during heat stress in durum wheat (Paolacci et al.

1997). The present results indicate the role of GSH and hmGSH in the response to heat stress in wheat.

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