THE EFFECT OF WATER DEFICIT ON THE ACTIVITY OF HYDROGEN PEROXIDE-SCAVENGING ENZYMES IN TWO BARLEY GENOTYPES

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ABSTRACT

Two barley (*Hordeum vulgare* L.) genotypes, the cv. Aramir and line R567, were subjected to water deficit by immersing their root systems in polyethylene glycol solution of osmotic potential -1.0 MPa. The stress caused a decline in the leaf-relative-water content (RWC) and affected membrane damage in both the genotypes. A higher decline in RWC and a higher membrane injury index was observed in R567 in comparison to 'Aramir'. Water deficit induced an increase in the activity of guaiacol peroxidase (GPO) and catalase (CAT). A higher increase of CAT than GPO peroxidase activity has been noted in both the genotypes.

The results, together with our earlier reports (Bandurska et al. 1997) show that detoxification of hydrogen peroxide under water stress conditions in those two barley genotypes was associated with the action of GPO and CAT, and that the latter was more involved in that process.

KEY WORDS: barley, catalase, guaiacol peroxidase, membrane injury, water deficit.

INTRODUCTION

Negative effect of water deficit on plant metabolism is associated with the generation of reactive oxygen species (Allen 1995), which include superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen (Smirnoff 1993). Plants are protected against the generation of reactive oxygen species with an array of small molecular antioxidants (ascorbic acid, glutathione, α -tocopherol, carotenoids) and antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxidase (APO) (Smirnoff 1993; Foyer et al. 1994; Nott and Foyer 1998). Peroxidases using other electron donors like guaiacol are also involved in this process (Cakmak et al. 1993). Several authors have shown that water stress induced an increase in hydrogen peroxide content and consequently caused membrane injury in plant tissues (Mukherje and Choudhuri 1983; Chowdhury and Choudhuri 1985). However, we have previously reported, that water deficit in barley genotypes ('Aramir', line R567), which caused membrane injury in the leaves also caused a decrease in the level of hydrogen peroxide and no increase in APO activity (Bandurska et al. 1997). The present study was carried out to examine in those two barley genotypes the effect of water deficit on the activity of two other H_2O_2 scavenging enzymes as CAT and GPO.

MATERIALS AND METHODS

Plant material and growth conditions

Barley seedlings (*Hordeum vulgare* L. cv. Aramir and line R567) were grown in an aerated nutrient solution under controlled conditions, according to Bandurska and Gniazdowska-Skoczek (1995). Six-day-old seedlings were used for the experiments.

Water deficit stress application and sample collection

The root system of plants was immersed in polyethylene glycol solution (PEG 6000) of osmotic potential -1.0 MPa (23.8% w/v) for 24 hours. Control seedlings were left in nutrient solution. The seedlings were kept under controlled conditions at 21°C, 70% relative humidity and continuous light of $140 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of photosynthetic photon flux density. After 2, 10 and 24 h of the stress, the leaves from seedlings of each set were harvested and used for analysis. The middle sections of the leaves were cut into 2 cm pieces immediately after sampling. Nine pieces from 3 leaves were placed in a 50 ml flask to determine membrane injury. Two leaf fragments were collected for RWC determination and 100-200 mg samples were collected for determination of CAT and GPO activities.

Determination of relative water content (RWC)

The level of water stress in leaves was estimated on the basis of relative water content (RWC) determined by Wea-

therly (1950) method according to Bandurska (1991). The RWC was calculated by the following formula: $RWC = (\text{fresh weight} - \text{dry weight}) / (\text{fresh weight of full turgor} - \text{dry weight}) \times 100\%$.

Determination of cell membrane injury

After stress treatment the leaf pieces, collected as described above, were washed quickly three times in 10 ml of deionized water, then immersed in 10 ml of deionized water and kept for 24 h at 10°C. Membrane injuries were determined as reported previously (Bandurska et al. 1997) and expressed as the percentage injury index following the formula of Sullivan (1971): $I = [1 - (1 - T_1 / T_2) / (1 - C_1 / C_2)] \times 100\%$, where C_1 and C_2 represent conductivity measurements of control samples before and after autoclaving, respectively; T_1 and T_2 represent conductivity measurements of water-stressed samples before and after autoclaving, respectively.

Enzymes extraction and assay

The leaf tissue was homogenized in 2 ml of 50 mM phosphate buffer pH 7.8 containing 1% polyvinylpyrrolidone and

centrifuged at $15\,000 \times g$ for 20 min. These operations were carried out at 4°C. The supernatant was used for analysis.

CAT (EC 1.11.1.6) activity was determined by measuring the rate of H_2O_2 conversion to O_2 at a room temperature, using oxygen electrode Oxytec, RE K1-1 (Del Rio et al. 1977). The enzyme activity was expressed in catalytic units $\cdot g$ dry weight $^{-1} \cdot \text{min}^{-1}$.

GPO (EC 1.11.1.7) activity was determined according to Cakmak et al. (1993). The oxidation of guaiacol in the presence of H_2O_2 was measured as the increase in absorbance recorded at 470 nm. The enzyme activity was expressed as $\Delta A \cdot g$ dry weight $^{-1} \cdot \text{min}^{-1}$.

Presentation of data

For every investigated parameter 3-5 independent replications were conducted in each combination. The results are means of all the replications. Tuckey's test was applied to determine the significance of differences between the means at $P=0.05$. Bars in Figs 1 and 2 labelled with the same letters are not significantly different.

Fig. 1. The effect of water deficit (PEG -1.0 MPa) on (A) relative water content and (B) membrane injury index in two barley genotypes. Bars in the figure labelled with the same letters are not significantly different at $P=0.05$ based on Tuckey's test.

Fig. 2. The effect of water deficit (PEG -1.0 MPa) on the activity of (A) guaiacol peroxidase and (B) catalase in two barley genotypes. Bars in the figure labelled with the same letters are not significantly different at $P=0.05$ based on Tuckey's test.

RESULTS AND DISCUSSION

Relative water content (RWC) in leaves of the examined genotypes significantly decreased under water deficit conditions (Fig. 1A). RWC decline in both the genotypes was directly proportional to the duration of the applied stress. At the last date of the stress, 'Aramir' showed a higher RWC than R567. Water deficit in leaves caused membrane damage (Fig. 1B). The membrane injury index was higher in R567 than in 'Aramir'. These results are consistent with those of our earlier reports indicating that the line R567 shows a lower tolerance to water deficit than 'Aramir' in terms of membrane stability (Bandurska and Gniazdowska-Skoczek 1995; Bandurska et al. 1997; Bandurska and Floryszak-Wieczorek 2002).

Figure 2 shows the effect of water stress on the activity of hydrogen peroxide scavenging enzymes in examined barley genotypes. GPO activity in the leaves of unstressed plants was significantly higher in R567 than in 'Aramir' (Fig. 2A). Under water stress conditions GPO activity in 'Aramir' significantly increased after a 2 h stress and slightly but insignificantly after 10 and 12 h of the stress. In R567 it increased after a 2 h stress, remained at the same level after a 10 h stress, and then after 24 h of the stress it decreased to the level of the control combination. Water deficit caused an increase in the activity of CAT in the both genotypes. The pattern of this enzyme increase was different in the genotypes examined. The cultivar Aramir showed a two-fold increase of CAT activity following a 10 h stress period and a 2.5-fold increase after 24 h. The activity of CAT in the line R567 increased about two-fold following a 10 h stress and then suppressed after 24 h.

In our previous experiments we observed a decrease in the level of H_2O_2 under water deficit conditions both in 'Aramir' and in R567 (Bandurska et al. 1997). According to Asada (1992) the removal of this metabolite in plant tissue can be achieved through the action of ascorbate peroxidase. However, we did not find that water deficit caused any increase in the activity of that enzyme (Bandurska et al. 1997). Therefore, from the results presented here it seems that GPO and CAT may be involved in H_2O_2 scavenging in those two barley genotypes.

A regulatory role of GPO in H_2O_2 scavenging was also observed under conditions of other abiotic stress factors, such as nickel toxicity in wheat (Pandolfini et al. 1992) and environmental pollution in Scots pine (Pukacka, Pukacki 2000).

The increase in CAT activity under water deficit conditions was also reported in wheat genotypes (Navari-Izzo et al. 1993; Sairam and Srivastava 2001). Some authors suggested that the rise in CAT activity under stress conditions is associated with an increase in stress tolerance, because it represents a potential defence mechanism against oxidative stress causing membrane damage (Prasad et al. 1994; Anderson et al. 1995; Kraus et al. 1995). The results obtained in the present paper show also clearly that CAT may play a regulatory role in scavenging H_2O_2 under water stress conditions. Moreover, the higher increase of CAT than GPO activity proves that the former was more involved in that process.

Sairam and Srivastava (2001) observed that under water stress conditions in wheat genotypes higher CAT activity was associated with lower accumulation of hydrogen peroxide and lower membrane injury. From the here presented

results it follows that a higher and more persistent increase of CAT activity ('Aramir') under stress conditions was also associated with a lower membrane injury (Fig. 1B and 2). However, taking into account that water deficit has caused a decrease in the level of leaf H_2O_2 in both the examined genotypes (Bandurska et al. 1997), it is rather unlikely that a higher CAT activity in 'Aramir' could be responsible for a lower membrane injury in that genotype.

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AKTYWNOŚĆ ENZYMÓW ROZKŁADAJĄCYCH NADTLENEK WODORU W LIŚCIACH DWÓCH GENOTYPÓW JĘCZMIENIA W WARUNKACH DEFICYTU WODY U DWÓCH

STRESZCZENIE

Siewki dwóch genotypów jęczmienia (*Hordeum vulgare*), odmiany Aramir i linii R567, poddano działaniu deficytu poprzez zanurzenie korzeni w roztworze glikolu polietylenowego (PEG) o potencjale osmotycznym -1.0 MPa. U obu genotypów stres spowodował obniżenie względnej zawartości wody (RWC) w liściach oraz uszkodzenie błon komórkowych. Większe odwodnienie liści oraz większe uszkodzenie błon komórkowych stwierdzono u linii R567 niż odmiany Aramir.

Deficyt wody spowodował wzrost aktywności peroksydazy guajakolowej (GPO) oraz katalazy (CAT). U obu genotypów stwierdzono większy wzrost aktywności CAT niż GPO.

Przedstawione wyniki oraz wyniki uzyskane we wcześniejszych badaniach (Bandurska i in. 1997) wskazują, że detoksykacja nadtlenu wodoru w warunkach stresu wodnego u badanych genotypów odbywa się przy udziale CAT i GPO, oraz że CAT odgrywa większą rolę w tym procesie.

SŁOWA KLUCZOWE: deficyt wody, jęczmień, katalaza, peroksydaza guajakolowa, uszkodzenia błon.