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HEAT SHOCK INFLUENCES THE ACTIVITY OF CATALASE, ASCORBATE PEROXIDASE AND GRAIN YIELD IN SPRING BARLEY (*Hordeum vulgare* L.)

Agnieszka Płażek, Ewa Pociecha, Renata Bączek-Kwinta

Department of Plant Physiology, Agricultural University, Kraków

Introduction

The exposure of plants to higher than optimal temperatures reduces the yield and decreases the quality of cereals. Heat stress induces the decrease of the duration of developmental phases, reduces light perception over the shortened life cycle, and causes perturbation of transpiration, photosynthesis and respiration. Especially, heat shock causes the disturbance in electron transport within thylacoid and mitochondrial membranes, which leads to the generation of reactive oxygen species (ROS) [DAVIDSON, SCHIESTL 2001; MAESTRI et al. 2002]. A consequence of elevated temperature in plants is the oxidative damage [FITTER, HAY 1987]. The accumulation of ROS limits the activity of several enzymes and causes the disturbance to the membrane structure and function [ALSCHER et al. 1997]. Moreover, high concentration of ROS declines the generation of ATP and redox metabolites that are essential to cellular defence and repair [FOYER 1997]. The overproduction of ROS could limit the plant tolerance to different stresses [DOKE et al. 1994]. The antioxidant defence mechanism is a part of heat stress adaptation and is strongly correlated with the thermotolerance. The activation of antioxidant enzymes usually is initiated by a significant increase in the reactive oxygen species (ROS) pool. Especially, the capacity to acquire thermotolerance is associated with the activities of catalase and ascorbic acid content [SAIRAM et al. 2000].

The goal of the presented work was to investigate if a heat shock (42°C) lasting 1 or 3 hours modifies the activities of catalase and ascorbate peroxidase and changes the grain yield of spring barley (*Hordeum vulgare* L.) cv. 'Sezam'.

Material and methods

Seeds of spring barley cv. 'Sezam' were sown in plastic pots, containing a mixture of soil : peat : sand (2 : 2 : 1 v/v/v) at pH 5.8 and grown at 18°C for 7 weeks in a phytotronic chamber at the photosynthetic photon flux density (PPFD) of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with 16 h photoperiod. Plants treated with high tem-

perature were in the tillering phase. The temperature was increased gradually for 2 h up to 42°C and lasted for 1 or 3 h. After this time the temperature was reduced back to 18°C. Control plants were kept continuously at 18°C. The activities of catalase (CAT) and ascorbate peroxidase (APX) were measured in the third full developed leaf before heating and after 2, 24 and 72 h of recovery from the heat shock. All control measurements were taken before the heat shock supposing that activity of studied enzymes do not change in the control conditions during three days.

Assay of catalase (CAT) (EC 1.11.1.6)

CAT activity was estimated according to AEBI [1984]. CAT activity is assayed in the reaction mixture (3 cm^3 final volume) composed of 50 mM phosphate buffer at pH 7.5 to which 30% (w/v) H_2O_2 is added to reach an absorbance value in the range of 0.520–0.550 (λ = 240 nm). The reaction was started after adding of 200 μl of crude extracts to the reaction mixture. CAT activity was expressed as a decrease in absorbance at 240 nm (using spectrophotometer LKB Ultrospec II) as a consequence of H_2O_2 consumption [AEBI 1984]. The decrease in absorbance of 0.0145 responded to 1 μmol H_2O_2 decomposed by CAT. Activity of the enzyme expressed as μmol H_2O_2 was related to protein content. Protein concentration was determined according to BRADFORD [1976] using the Bio-Rad (Munich, Germany) protein assay with BSA as a calibration standard. Assays were done in 5 different leaves collected from different plants.

Assay of ascorbate peroxidase (APX) (EC 1.11.1.11.)

Ascorbate peroxidase was measured spectrophotometrically according to NAKANO and ASADA [1981]. Leaf samples were ground to a fine powder with liquid nitrogen and extracted with 50 mM phosphate buffer (pH 7.0) and 1 mM EDTA (SIGMA), then centrifuged at 14 000 rpm. One cm^3 of reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.25 mM ascorbic acid and 0.5 mM H_2O_2 was mixed with 10 μl of crude extract. Absorbance was measured at 290 nm. Peroxidase activity was expressed as μmol of ascorbic acid·min^{-1}·mg^{-1} protein. Assays were done in 5 different leaves collected from different plants.

Measurement of grain yield

After five weeks of recovery from heat shock the yield of ripe grain was determined in 100 ears from each studied plant group. Then, the number of grain per one ear was calculated.

Statistical analyses

The effect of heat shock on the studied parameters was tested with *F*-test (analysis of variance ANOVA, Statistica 5.0). The results were analysed using Duncan's multiple range test at $\alpha < 0.05$.

Results

One-hour heat shock initially (after 2 h of recovery from heat shock) decreased CAT activity, while after 72 h of recovery from heat shock CAT activity increased in the comparison with the control plants (Fig. 1). Three-hour stress, similarly to 1-hour treatment, caused the decrease in activity of this enzyme, but a greater activation occurred after 24 h of recovery, thus faster in comparison with the effect of 1-hour heat shock. One-hour treatment with temperature of 42°C increased APX activity after 24 h and caused its decrease after 72 h of recovery (Fig. 2). Three-hour heat shock increased APX activity 2 h after return to 18°C, and later (after 24 h) its decrease. Both 1-hour and 3-hour treatment with 42°C caused a significant decrease in the number of grains, however, no considerable effect of stress time on the grain yield was stated (Tab. 1).

Table 1; Tabela 1

The influence of treatment of 7-week-old plants of spring barley 'Sezam' cv. with 42°C for 1 and 3 hours on the average number of seeds obtained from one ear (the means marked with the same letters do not differ significantly – Duncan test $\alpha < 0.05$)

Wpływ traktowania temperaturą 42°C przez jedną lub trzy godziny na średnią liczbę zawiązanych ziarniaków w jednym kłosie jęczmienia jarego odm. 'Sezam' (średnie zazanaczone tą samą literą nie różnią się istotnie – test Duncana $\alpha < 0,05$)

1-hour-stress; 1 godz.		3-hour-stress; 3 godz.	
control; kontrola	heated; traktowane wysoką temperaturą	control; kontrola	heated; traktowane wysoką temperaturą
15.7a	10.7b	16.3a	10.1b

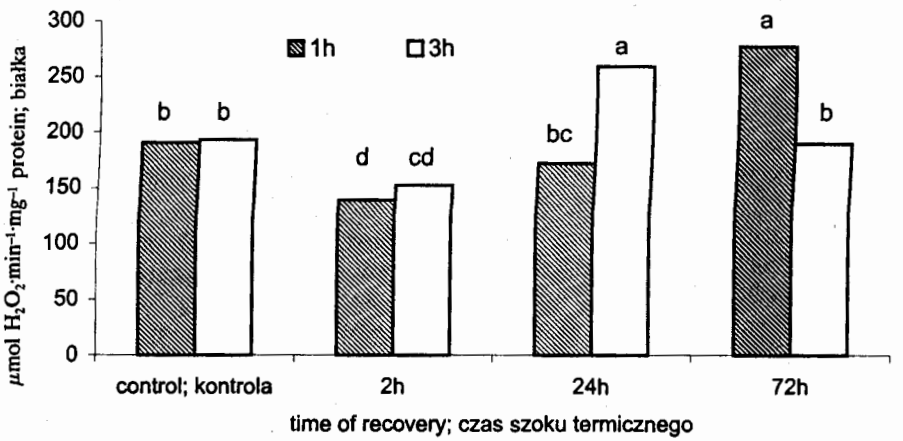

Fig. 1. CAT activity on barley leaves treated with the temperature of 42°C for 1 or 3 hours. The objects marked with the same letters do not differ significantly (Duncan test $\alpha = 0.05$)

Rys. 1. Aktywność katalazy (CAT) w liściach roślin jęczmienia traktowanych temperaturą 42°C przez 1 lub 3 godziny. Średnie zaznaczone tą samą literą nie różnią się statystycznie istotnie (test Duncana $\alpha = 0,05$)

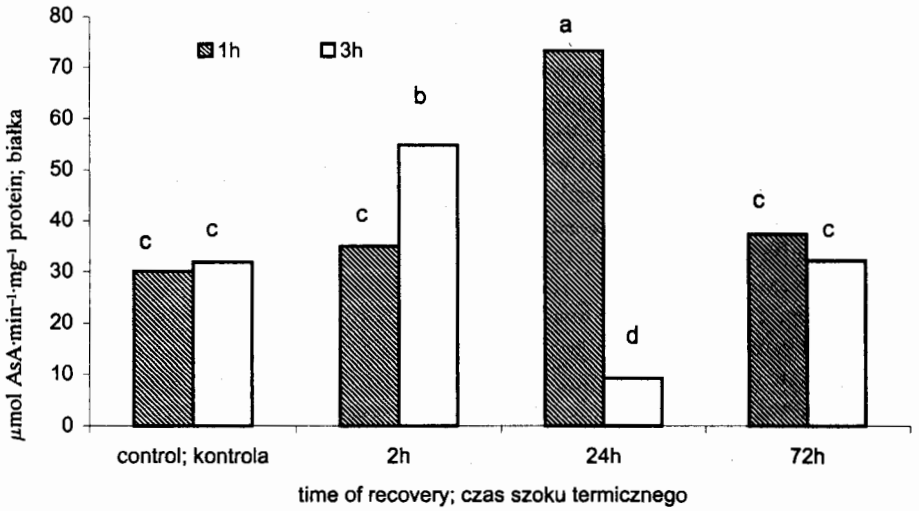

Fig. 2. APX activity on barley leaves treated with the temperature of 42°C for 1 or 3 hours. The objects marked with the same letters do not differ significantly (Duncan test $\alpha = 0.05$)

Rys. 2. Aktywność peroksydazy askorbinianowej (APX) w liściach roślin jęczmienia traktowanych temperaturą 42°C przez 1 lub 3 godziny. Średnie zaznaczone tą samą literą nie różnią się statystycznie istotnie (test Duncana $\alpha = 0,05$)

Discussion and conclusions

Heat shock is a form of the oxidative stress, resulting in the formation of many toxic ROS in plants [EL-SHINTINAWY et al. 2004]. A consequence of an increase in ROS pool is the activation of the antioxidant system. SAIRAM et al. [2000] stated that the increase in the antioxidant enzyme activity was strongly correlated with the heat stress adaptation of wheat plants. PANCHUK et al. [2002] showed, that in Arabidopsis APX activity increased by severe stress at 44°C. In the presented study the pattern of the plant response under the heat shock manifested by a rapid increase in CAT and APX activities depended on stress duration. Three-hour treatment with 42°C accelerated barley reaction (activation of CAT and APX) in comparison with one-hour stress. This reaction could be associated with a greater increase of hydrogen peroxide concentration in cells after three hours of heat shock than during treatment with the same temperature for only one hour.

The extent of damage caused by exposure to a high temperature may differ remarkably depending on the stage of growth and type of plant tissue. The temperature threshold for damage by high temperatures in reproductive organs is considerably lower than in other crop organs. Pollen viability and growth is one of the most heat-sensitive of these developmental stages in cereals [STONE 2001]. In maize, the reduction in seed set occurs at a temperature higher than 38°C mainly due to the reduction in pollen germination ability and tube elongation [DUPUIS, DUMAS 1990; STONE 2001]. The temperature above 20°C decreases final mass of wheat seedlings, while phasic development accelerates. This leads to reduced final yields even when the rest of the season temperatures are at the optimum. In maize, root and shoot biomasses declined by 10% per degree above

26°C [after STONE 2001]. In the presented experiments even one-hour heat stress decreases the grain yield of barley. It should be marked that the heat shock did not affect plants during flowering, but probably in the early developmental phase of inflorescence (morphological development was not stated). It could be supposed that ROS damaged the primordia of spikelets during their early development.

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Key words: barley, defence response, high temperature stress, oxidative stress

Summary

In crops the temperature of 42°C causes a lost of yield, disturbance in light phase of photosynthesis and electron transport in mitochondrium membranes. These effects could be caused also by reactive oxygen species generated as the aftermath of heat shock. The aim of the work was to estimate if 42°C for 1 or 3 h influences grain number and activity of antioxidant enzymes in leaves of spring barley.

Plants of spring barley cv. 'Sezam' were grown at 18°C for seven weeks. Next, temperature increased for 2 h up to 42°C and remained for 1 or 3 h. After this time temperature declined back to 18°C. Control plants were kept at 18°C. The activities of catalase (CAT) and ascorbate peroxidase (APX) were measured before heat shock and 2, 24 and 72 h of recovery. CAT activity was estimated according to AEBI [1984], while APX activity according to NAKANO and ASADA [1981]. After the next 5 weeks the yield of rape grain was calculated.

One-hour-heat shock increased APX activity after 24 h, while CAT activity after 72 h of recovery. Three-hour-heat shock caused first (after 2 h) increase, and later (after 24 h) decrease in APX activity. In this case of stress-treatment CAT activity increased in 24 h after the return to 18°C. Both 1 h and 3 h of 42°C caused a significant decrease in the number of grains.

SZOK CIEPLNY WPŁYWA NA AKTYWNOŚĆ KATALAZY, PEROKSYDAZY ASKORBINIANOWEJ ORAZ NA PLON ZIARNIAKÓW JĘCZMIENIA JAREGO (*Hordeum vulgare* L.)

Agnieszka Płażek, Ewa Pociecha, Renata Bączek-Kwinta
Katedra Fizjologii Roślin, Akademia Rolnicza im. H. Kołłątaja w Krakowie

Słowa kluczowe: jęczmień, reakcja odpornościowa, stres wysokotemperaturowy, stres oksydacyjny

Streszczenie

Temperatura 42°C powoduje spadek plonu roślin uprawnych, zakłócenia fazy jasnej fotosyntezy i transportu elektronów w błonach mitochondrialnych. Przyczyną wymienionych efektów może być utleniające działanie reaktywnych form tlenu (RFT) akumulowanych w dużych ilościach w następstwie szoku termicznego. Celem prezentowanej pracy było zbadanie, czy szok cieplny (42°C) trwający jedną lub trzy godziny wpływa na liczbę ziarniaków w kłosach oraz aktyw-

ność enzymów antyoksydacyjnych w liściach jęczmienia jarego.

Rośliny jęczmienia jarego odmiany 'Sezam' wzrastały w 18°C przez siedem tygodni. Następnie temperaturę podnoszono stopniowo przez dwie godziny do 42°C i utrzymywano ją na tym poziomie przez jedną lub trzy godziny. Po tym czasie temperaturę obniżano stopniowo przez dwie godziny do 18°C. Próbki do oznaczenia aktywności katalazy (CAT) i peroksydazy askorbinianiowej (APX) pobierano po 2, 24 i 72 godzinach po obniżeniu temperatury do wartości kontrolnej. Aktywność CAT oceniano według metody AEBI [1984], podczas gdy aktywność APX oznaczano zgodnie z procedurą NAKANO i ASADY [1981]. Po upływie następnych pięciu tygodni oceniano średnią liczbę dojrzałych ziarniaków zebranych z jednego kłosa.

Jednogodzinny szok termiczny powodował wzrost aktywności APX po 24 godzinach, oraz wzrost aktywności CAT po 72 godzinach po obniżeniu temperatury do wartości kontrolnej. Temperatura 42°C utrzymująca się przez trzy godziny powodowała najpierw (po 2 godzinach) wzrost, a następnie (po 24 godzinach) spadek aktywności APX, oraz wzrost aktywności CAT po 24 godzinach po ustaniu stresu. Jedno- i trzygodzinny stres wysokotemperaturowy powodował istotny spadek średniej liczby ziarniaków zebranych z jednego kłosa.

Dr hab. Agnieszka **Płażek**
Katedra Fizjologii Roślin
Akademia Rolnicza im. H. Kołłątaja
ul. Podłużna 3
30–239 KRAKÓW
e-mail: rrplazek@cyf-kr.edu.pl