Acta Physiologiae Plantarum (2019) 41:137
https://doi.org/10.1007/s11738-019-2930-x

ORIGINAL ARTICLE

Responses of young wheat plants to moderate heat stress

Tibor Janda[1] · Radwan Khalil[2] · Judit Tajti[1] · Magda Pál[1] · Éva Darkó[1]

Received: 7 February 2019 / Revised: 3 July 2019 / Accepted: 11 July 2019 / Published online: 17 July 2019

Abstract

Moderate heat stress may provide protection against a subsequent severe high temperature stress in plants. However, the exact mechanisms of heat acclimation of wheat are still poorly understood. In the present work, two wheat varieties Ellvis and Soissons were exposed to a moderate elevated temperature at 30 °C, and the changes of certain protective mechanisms were investigated. Although the differences in the proline level between the genotypes were not substantial, it was approx. 2–3 times higher in the heat-treated plants than in the controls. After exposure to moderate elevated temperature, the activities of ascorbate peroxidase and catalase were also induced. Similarly, the amount of the free salicylic acid also increased after moderate heat stress, independently on the genotypes. The amount of the main polyamines, namely, putrescine, spermidine, and spermine either did not change or decreased after the same period. However, heat acclimation increased the level of 1,3-diaminopropane, in parallel with a polyamine oxidase gene, *TaPAO*. While the expression level of the peroxisomal polyamine oxidase gene *Ta*perPAO hardly changed, *Ta*PAO showed a substantial increase after 1 day, especially in Soissons, and at the end of the heat treatment was still significantly higher than in the controls. These suggest that signalling processes related to polyamine metabolisms or salicylic acid-related processes might also contribute to the higher heat tolerance induced by moderate heat stress. The variations in recorded measurements were mainly temperature dependent, and the effect of genotype was less pronounced than the effect of moderate heat treatment itself.

Keywords Abiotic stress · Antioxidant activity · Heat acclimation · Moderate heat stress · Polyamine cycle · *Triticum aestivum* l.

Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
DAP	1,3-Diaminopropane
G-POD	Guaiacol peroxidase
GR	Glutathione reductase
GST	Glutathione-*S*-transferase
*o*HCA	*Ortho*-hydroxy-cinnamic acid
PAO	Polyamine oxidase
PUT	Putrescine
qRT-PCR	Quantitative real-time PCR
SA	Salicylic acid
SPD	Spermidine
SPDS	Spermidine synthase
SPM	Spermine
TaPAO	Putative apoplastic polyamine oxidase gene
TaperPAO	Peroxisomal polyamine oxidase gene

Communicated by U. Feller.

✉ Tibor Janda
janda.tibor@agrar.mta.hu

[1] Agricultural Institute, Centre for Agricultural Research of the Hungarian Academy of Sciences, Brunszvik u. 2, Martonvásár 2462, Hungary

[2] Botany Department, Faculty of Science, Benha University, Benha 13518, Egypt

Introduction

Heat stress is one of the most important abiotic stress factors limiting growth of crop plants in many regions of the world leading to dramatic reduction in the economic yield (Battisti and Naylor 2009). Wheat plants, especially winter varieties are usually less adapted to warmer temperatures than other main crops, such as maize or rice. Worldwide, heat stress can reduce the yield of wheat by as much as 15% (Qin et al. 2008). Global climate change has also exacerbated the severity of many abiotic stresses, including temperature extremes, with significant yield reductions in several crop plants (Huang et al. 2018). Plants have evolved effective mechanisms to acclimate to the variable environment.

However, when these changes are rapid and extreme, they are often unable to avoid or significantly mitigate stress. This is especially true for crop plants, which often originate from far regions with different climate and have relatively narrow genetic diversity to cope with the fluctuating environment. Better understanding of stress adaptation mechanisms may also provide useful information for breeders for developing new crops with better stress tolerance.

In general, responses to high temperatures may involve, among others, alteration of transcriptional control, synthesis of osmoprotectants, heat shock proteins (Hasanuzzaman et al. 2013), modifying carbohydrate metabolism (Wang et al. 2012) or the induction of various signalling processes (Proveniers and van Zanten 2013), and antioxidative systems to reduce the harmful effects of oxidative damage (Zhou et al. 2019). Exposure of plants to non-lethal, slightly elevated temperatures may provide protection against a subsequent severe high temperature stress (heat acclimation). The mechanisms of heat acclimation of the photosynthetic machinery have been extensively studied in various crop species, including wheat. The effectiveness of heat acclimation depends on various factors. For example, it also shows a season dependence, as it was recently demonstrated in winter wheat plants (Zhou et al. 2018).

Polyamines (PAs), such as putrescine (PUT), spermidine (SPD), and spermine (SPM), can be found in relatively high amounts in all living cells, and their protective role has been demonstrated under various stress conditions (Pál et al. 2015, 2018a, b). Besides their direct protective role, they also regulate fundamental cellular processes as signalling molecules. Recent results suggest that stress tolerance induced by the different PAs is mainly related to their signalling role rather than to their accumulation (Alcázar et al. 2010; Pál et al. 2015, 2019; Szalai et al. 2017). However, the involvement of the PA cycle in the heat-acclimation processes has not been characterised yet.

It has been shown that the differences in heat tolerance in different wheat genotypes may be associated with multiple mechanisms regulated by various transcription factors and involving several stress-related genes (Qin et al. 2008). However, in spite of the extensive research on this field, the molecular mechanisms of the heat-acclimation processes, especially in cereals, are still poorly understood. We have recently demonstrated that wheat varieties of different origins could be efficiently heat-acclimated when plants were exposed to 30 °C for a certain period (Végh et al. 2018). Growing at this acclimating temperature did not induce either substantial stomatal closure or photoinhibition. Certain genotypes were able to induce transpiration at this temperature and did not reduce net assimilation. Using different heat sensitivity and heat-acclimation indexes, it was possible to differentiate the heat responses of the different wheat genotypes (Perdomo et al. 2015; Végh et al. 2018).

Using our previously described heat-acclimation system in wheat (Végh et al. 2018), the next goal was to further evaluate the effects of moderate heat stress in these plants. The present work aimed at identifying processes with substantial changes after heat treatment, and those with variation between the genotypes with different levels of heat tolerance. In the present study, we focused on certain stress acclimation mechanisms, which may play decisive role in various stress responses. These included the accumulation of proline or salicylic acid and induction of antioxidant enzymes. Special attention has been focused on PA signalling and metabolisms both at metabolite and gene expression levels.

Materials and methods

Plant materials and growth conditions

Wheat (*Triticum aestivum* L.) genotypes var. Ellvis and Soissons were grown in pots filled with 3:1 (v:v) mixture of loamy soil and sand in a Conviron PGV-15 growth chamber (Controlled Environments Ltd., Winnipeg Canada) at 20/18 °C day/night temperature with 16/8 h photoperiod at 250 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the canopy level, and 75% relative humidity. 10 days after sowing (young plants with 2–3 leaves) part of the plants were heat-treated at 30/27 °C day/night temperature, while others were grown at 22/20 °C (controls) for 14 days. Samples were taken and measurements were carried out at different days indicated at the measurements.

Determination of proline content

The determination of proline content was based on its reaction with ninhindrin according to the methods of Bates et al. (1973). In the experiments, 0.5 g of leaf sample was extracted with 5 ml 3% sulfosalicylic acid (w/v). After centrifugation, 1 ml supernatant was reacted with 1 ml ninhydrin and 1 ml of glacial acetic acid for 1 h at 100 °C. The chromophore was extracted with 4 ml toluene from the cooled reaction mixture, and the absorbance of chromophore was read at 520 nm, using toluene for a blank. The proline concentration was determined from a standard curve and calculated as mg proline/FW.

Measurements of antioxidant enzymes

For the analysis of antioxidant enzyme activity, 0.5 g leaf tissue was homogenized in 2.5 ml ice-cold Tris buffer (0.5 M, pH 7.5) containing 3 mM $MgCl_2$ and 1 mM EDTA. The enzyme activities were determined photometrically with a UV–visible recording spectrophotometer (UV–Vis 160A,

Shimadzu Corp. Kyoto, Japan), and expressed in nkat g^{-1} fresh weight (FW). The ascorbate peroxidase (APX; EC 1.11.1.11.) and the catalase (EC 1.11.1.6.) activities were measured as described earlier (Janda et al. 1999). The guaiacol peroxidase (G-POD; EC 1.11.1.7.), the glutathione reductase (GR; EC 1.6.4.2.), and the glutathione-*S*-transferase (GST; EC 2.5.1.18.) activities were determined according to Ádám et al. (1995), Smith et al. (1988), and Mannervik and Guthenberg (1981), respectively.

Determination of salicylic acid and *ortho*-hydroxi-cinnamic acid

Salicylic acid (SA) extractions were performed according to Pál et al. (2005) by grinding 0.5 g of plant tissue in liquid nitrogen in a mortar and pestle, in the presence of 0.5 g quartz sand. After separation on a reverse phase column (Synergi Fusion-RP, 80A, 150×4.6 mm, 4 µm, Phenomenex, Inc. California, USA) SA and *ortho*-hydroxy-cinnamic acid (*o*HCA) were quantified fluorimetrically (W474 scanning fluorescence detector, Waters, Milford, USA), with excitation at 317 nm and emission at 436 nm for *o*HCA, followed by excitation at 305 nm and emission at 407 nm for SA.

PA and 1,3-diaminopropane analyses

The analyses were carried out as described earlier (Németh et al. 2002) with slight modifications. 200 mg of leaves were homogenized with 1 ml 0.2 M ice-cold perchloric acid and were left to stand for 20 min on ice. The extract was centrifuged at 10,000*g* for 20 min and the supernatant was used. PUT, SPD, and SPM, together with the catabolit of the latter two ones, 1,3-diaminopropane (DAP) were analysed as dansylated derivatives via HPLC using a W2690 separation module and a W474 scanning fluorescence detector with excitation at 340 nm and emission at 515 nm (Waters, Milford, MA, USA).

Real-time PCR

Total RNA was extracted from fully developed leaf and root samples using TRI Reagent®. The samples were treated with DNase I and cleaned with a Direct-zol™ RNA MiniPrep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. cDNA synthesis was carried out using M-MLV Reverse Transcriptase (Promega Corporation, Madison, WI, USA). The candidate genes encoding for PAO were identified with RNAseq analysis according to Xiong et al. (2017). The presence of peroxisomal target signal of the candidate gene Q4 was analyzed via PPero PTS1 Type Peroxisomal Protein Prediction Model (Wang et al. 2017). Gene-specific primers and housekeeping primers (Table 1), PCRBIO SyGreen Mix (PCR Biosystems, London, UK), and CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) were used for quantitative real-time PCR (qRT-PCR) reaction. The relative gene expression values were determined with the ΔΔCt method (Livak and Schmittgen 2001). Ct values were normalized by the Ct values of housekeeping gene *Ta2291* encoding for an ADP-ribosylation factor. All reactions were performed in triplicate.

Statistical analyses

Usually, five parallel samples were collected for each measurements. Significant differences between the treatments and the genotypes were probed using the *t* test method.

Results

Effects of moderate heat stress in young wheat plants

Under control conditions, slightly, higher proline level could be detected in the leaves of young Ellvis than in Soissons (Fig. 1). Exposure to acclimating temperature at 30 °C for 14 days significantly increased, approx.

Table 1 Reference gene and target genes investigated in wheat plants using qRT-PCR

Gene name	Primer sequences (5′→3′)	Reference
Ta2291 (ADP-ribosylation factor)	F: GCTCTCCAACAACATTGCCAAC R: GCTTCTGCCTGTCACATACGC	Paolacci et al. (2009)
Traes_7AL_425787F27 (Q2;TaPAO) (putative apoplastic PAO)	F: CCAGCCTCCAGCTCCGCAAC R: GCCCAGCTCCTCCACCTCGTC	Xiong et al. (2017)
Traes_2BL_DA615F345 (Q4;TaperPAO) (putative peroxisomal PAO)	F: GCTCATAAATCAGCCCAATTCCA R: TTCGCCATTTGTTGAGCTCT	

F forward, *R* reversed primers

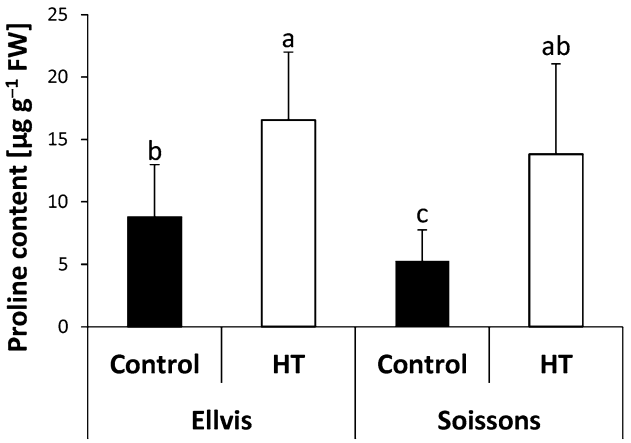

Fig. 1 Proline contents in the leaves of control (growing at 20/18 °C) and in heat-treated (2 weeks at 30 °C; HT) young wheat Ellvis and Soissons plants. Error bars indicate standard deviations. Different letters on the bars represent statistically significant differences between the mean values at $p < 0.05$ level

doubled the proline level in both genotypes. In the heat-treated plants, the difference between the varieties was not significant.

To better understand the effects of moderate heat stress on the antioxidant capacity, five antioxidant enzymes, namely, APX, GR, CAT, G-POD, and GST, were measured. Among these neither the genotype nor the elevated temperature significantly affected the activities of GR and G-POD enzymes (data not shown). The most pronounced changes occurred in the activities of APX. Its activity was significantly (ca. 50%) higher in the heat-treated plants than in the controls, but there was no significant difference between the genotypes (Fig. 2a). Catalase activity was higher in the Soissons than in Ellvis at control temperature. The moderate heat stress increased the catalase activity in Ellvis resulting in similar values, as was obtained in Soissons (Fig. 2b). The GST activities only showed a slight, but statistically significant increase after heat priming in Soissons (Fig. 2c).

The level of both free and bound (conjugated) forms of endogenous SA was also measured together with one of its putative precursors, *o*HCA. SA was mainly present in the leaves in bound form, either in the control or in the heat-treated plants (Fig. 3). Exposure to 30 °C for 14 days almost doubled the level of free and bound SA forms in both genotypes. Ellvis usually could be characterised with higher amount of free SA as compared to Soissons both under control and heat-acclimated conditions. However, these differences were not observed in the bound form. The free *o*HCA level did not differ between either the treatments or the genotypes, but the bound *o*HCA was

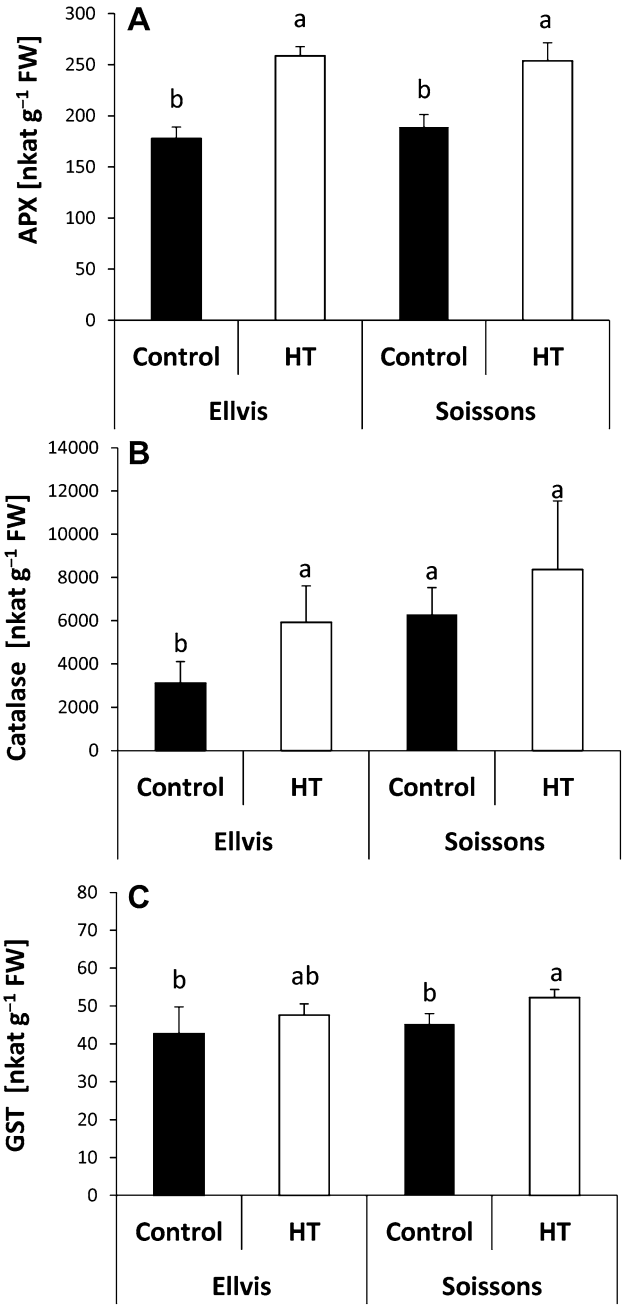

Fig. 2 Effects of moderate heat stress on the activities of ascorbate peroxidase (APX; **a**, catalase **b**, and glutathione-*S*-transferase (GST; **c**) enzymes in the leaves of young wheat plants, Soissons and Ellvis. Black bars: control plants growing at 20/18 °C; white bars: heat-treated (30 °C; HT) plants. Error bars indicate standard deviations. Different letters on the bars represent statistically significant differences between the mean values at $p < 0.05$ level

slightly (but statistically significantly) higher in the heat-treated Soissons than in the heat-acclimated Ellvis.

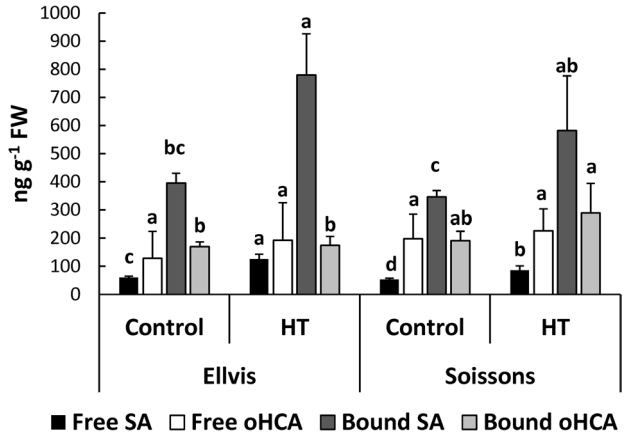

Fig. 3 Effects of moderate heat stress on the levels of free (black bars) and bound (dark grey bars) salicylic acid (SA) and on free (white bars) and bound *o*HCA (light gray bars) contents in the leaves of young control (growing at 20/18 °C) or heat-treated (30 °C; HT) Soissons and Ellvis wheat plants. Error bars indicate standard deviations. Different letters on the bars represent statistically significant differences between the mean values for each measured metabolite at $p < 0.05$ level

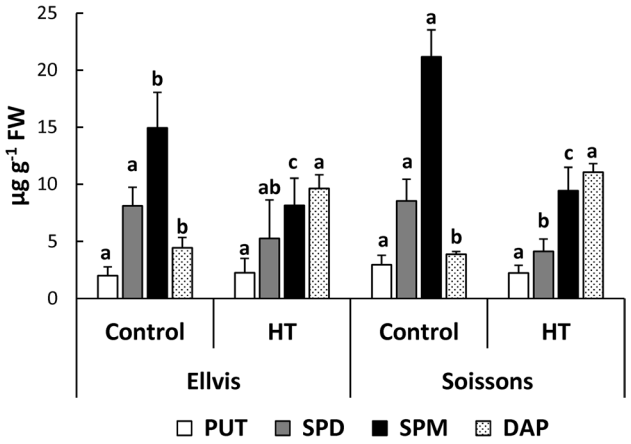

Fig. 4 Effects of moderate heat stress on the levels of putrescine (PUT; white bars), spermidine (SPD; grey bars), spermine (SPM; black bars), and 1,3-diaminopropane (DAP; dotted bars) contents in the leaves of young control (growing at 20/18 °C) or heat-treated (30 °C; HT) Soissons and Ellvis wheat plants. Error bars indicate standard deviations. Different letters represent statistically significant differences between the mean values for each measured metabolite at $p < 0.05$ level

Effects of moderate heat stress on PA metabolism in wheat plants

Under control conditions, SPM was the most abundant PA in both genotypes, and its level was slightly higher in Soissons than in Ellvis. The amount of SPM significantly decreased in the heat-treated samples and the original difference between the genotypes could not be detected. Similarly, the amount of SPD significantly decreased during the moderate heat stress, but it was statistically significant only in case of Soissons. The level of PUT did not differ significantly either between the genotypes or between the treatments. In contrast to the PUT, SPD, and SPM, the moderate heat stress resulted in a doubled accumulation of DAP, the catabolic product of SPM and SPD, in both genotypes (Fig. 4).

Since the conversion of SPD or SPM depends on the activity of PA oxidases (PAOs), the expression levels of two genes encoding two different types of PAOs, namely, *Ta*perPAO (peroxisomal localised PAO, mainly responsible for the back-conversion of SPM/SPD to PUT) and *Ta*PAO (putative apoplastic localised PAO, responsible for the production of DAP), have been determined not only at the end of the heat-treatment period (2 weeks), but also after 1 day at 30 °C. While the expression level of *Ta*perPAO hardly changed during the experiment (Fig. 5a), *Ta*PAO showed a substantial increase (> 25-times higher) after 1 day, especially in Soissons. At the end of the heat treatment, the expression level of *Ta*PAO was lower, but still significantly higher than in the control plants (Fig. 5b).

Discussion

We have previously described a heat-acclimation system, where wheat plants could be efficiently acclimated to elevated temperatures (Végh et al. 2018). In that work, a moderate heat stress at 30 °C was applied, which could be considered as heat acclimation showing positive effects during the subsequent heat stress responses of the photosynthetic processes. In this way, wheat Soissons was characterised with a relatively high heat-acclimating index, which indicated that this variety was a relatively heat tolerant genotype at young age. In contrast to this, Ellvis showed a high heat sensitivity (Végh et al. 2018). To better understand the acclimation mechanisms of these plants, in the present study, we used these genotypes with different heat tolerance, and a moderate heat stress was applied under the same experimental conditions as before (Végh et al. 2018).

Proline accumulation has been described in various plants exposed to different types of stressors, including unfavourable temperature conditions, either at low or high temperatures (Hayat et al. 2012; Majláth et al. 2012). Proline may act as a signalling molecule to regulate mitochondrial functions, and it also modulates redox balance and energy status (Szabados and Savouré 2010). Accumulation of proline or other osmolytes may also alleviate osmotic stress induced by heat stress (Li et al. 2018). Under the present experimental conditions, although the difference in the proline level between the wheat genotypes were not substantial, it was approx. 2–3 times higher in plants exposed to moderate heat stress at 30 °C than in the controls. This is in consistency

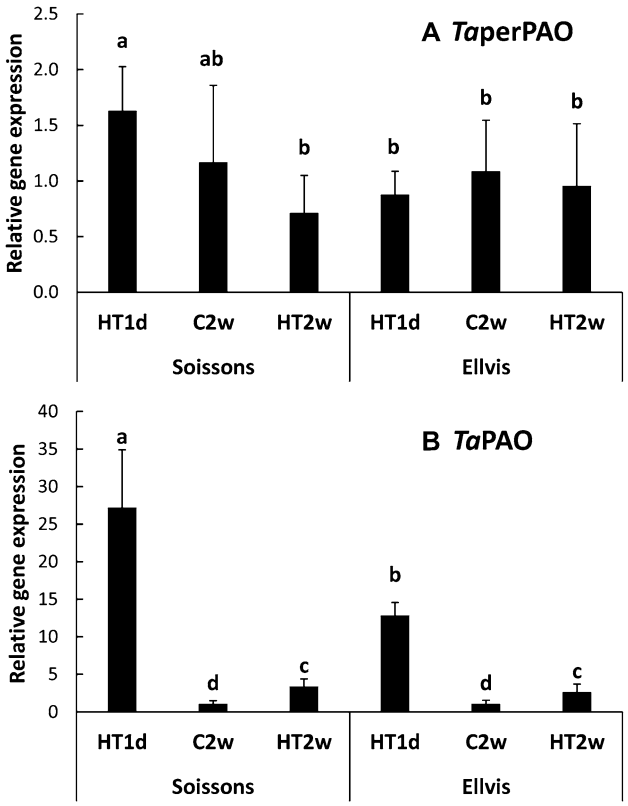

Fig. 5 Effects of moderate heat stress at 30 °C on the expression levels of *Ta*perPAO (**a**) and *Ta*PAO (**b**) in the leaves of young wheat plants, Soissons and Ellvis. Samples were collected after 1 day (1d) and 2 weeks (2w) during the heat-treatment period from control (C) and heat-treated (HT) plants. Control plants after 1 day were used as a reference with value 1 for each group. Error bars indicate standard deviations. Different letters represent statistically significant differences for each genes at $p < 0.05$ level

with other studies, where even much higher increase was reported in heat-exposed wheat plants (Khan et al. 2015a). In contrast to this, decrease in the proline levels has also been reported above a threshold temperature region, or after exposure of wheat plants to short-term heat shock (Song et al. 2005; Kumar et al. 2012). Our results supported that the present applied heat-treatment caused only a mild, stimulating stress. Furthermore, proline accumulation was much more affected by the environmental conditions and the physiological stage of the plants than by the genotype; therefore, proline can hardly be claimed as an indicator of heat tolerance.

Due to enhanced production of reactive oxygen species abiotic stresses are often accompanied with oxidative stress, which further damages several cell functions. Earlier studies have shown that heat stress acclimation processes also include antioxidant defence mechanisms, which are also associated with the acquisition of thermotolerance (Camejo et al. 2007; Zhao et al. 2014). Furthermore, in some cases, higher abiotic stress tolerance was assumed at least partially due to the higher constitutive antioxidant enzyme activities in the tolerant than in the sensitive plant genotypes (Zhao et al. 2011). Our results, based on the measurements of various antioxidant enzymes in control and heat-treated wheat plants, partly support that moderate heat stress can induce antioxidant defence mechanisms. After exposure to elevated, but non-lethal, acclimating temperature, the activities of certain antioxidant enzymes, such as APX and CAT, were induced. These enzymes play decisive role in the elimination of excess hydrogen peroxide accumulating under various stress conditions. However, although there were some differences between the wheat varieties investigated in our present work, the effects of moderate heat stress were more significant than the differences between the genotypes.

The activity of the antioxidant enzymes has also been regulated by several factors. SA is a phenolic compound, and it functions as a plant growth regulator. The role of SA in the regulation of various physiological processes, including stress responses has been widely reported (Pancheva and Popova 1998; Uzunova and Popova 2000; Janda et al. 2012). Studies have also shown that SA may alleviate the damaging effects of abiotic stressors, including low (Janda et al. 1999) or high temperatures (Dat et al. 1998; Larkindale and Knight 2002). The mode of action of SA in the induction of stress tolerance is complex. It may include induction of osmolites or antioxidant capacity, it may interact with other hormones (Khan et al. 2015b). In wheat plants exogenous SA also alleviated the adverse effects of heat stress on photosynthesis by mechanism of induced proline accumulation and interaction with ethylene (Khan et al. 2013). Similar to proline, free and bound SA also increased after exposure to moderate heat stress under the present experimental conditions, independently on the genotypes. However, the putative precursor, *O*hca, did not show substantial differences, in contrast to what was found during cold hardening period in wheat, where substantial increase in its bound form was reported (Janda et al. 2007). Besides its role in SA synthesis, *o*HCA has also been characterised as a potential antioxidant compound (Foley et al. 1999). Since neither the majority of the antioxidant enzymes nor the *o*HCA level were substantially higher at the end of the heat-acclimation period, these results suggest that, under the present experimental conditions, the protection against oxidative damage could not be the main mechanism of the acquired thermotolerance in these genotypes.

PAs are essential compounds playing role in various cell functions including responses to environmental stresses. Recent findings suggest that they are not only direct protective materials, but also function as signal triggering certain acclimation mechanisms. The interaction between PA metabolism and proline synthesis, SA signalling, or other stress-related processes has also been described (Pál et al. 2015, 2018b; Szalai et al. 2017). Some PAOs catalyse the

production of metabolic end-products of PAs, while some may catalyse the reverse reaction of PA synthesis in the PA cycle, the back-conversion of PAs (Pál et al. 2015; Chen et al. 2019). The conversion of PAs (PUT–SPD–SPM) is often described under stress conditions (Pál et al. 2015). To date, much less studies have focused on the molecular functions of PAs in plants under high temperature stress than under other stress conditions (Chen et al. 2019). Exogenous SPD could improve carbohydrate and nitrogen status of tomato seedlings at high temperatures through regulating the gene expression and activity of key enzymes for nitrogen metabolism (Shan et al. 2016). The present results showed that moderate heat stress in young wheat plants did not increase the levels of the major PAs, as PUT did not change, while the SPD and SPM decreased during the heat-treatment period. These results, in accordance with the slight changes of other metabolites and antioxidants, also supported that moderate heat stress under the present environmental conditions did not result in serious stress symptoms in the plants. Degradation of SPD by apoplastic localised PAO produces 1-pyrroline and DAP, whereas breakdown of SPM yields 1,3-aminopropylpyrroline and with DAP and hydrogen peroxide (Smith 1985; Bagni and Tassoni 2001). DAP can be further converted into alanine, while 1-pyrroline via γ-aminobutyric acid to succinate. Thus, PA catabolism is not simply a degradative process, but may also serves as a signal playing role in stress acclimation processes (Pál et al. 2015). In our experiments, the accumulation of DAP was mainly mediated by the *TaPAO* gene rather than *TaperPAO*, as induced expression was found only in the case of the former one. A substantial increase in the gene expression level was found after 1 day of the heat-acclimation period, then it was less pronounced after two weeks. This is in agreement with earlier findings, that heat acclimation has less effects on gene expression under prolonged treatments then at short-term heat stress in wheat (Qin et al. 2008). These results suggest that heat acclimation in young wheat plants does not involve the stress-induced accumulation of main PAs, rather though the terminal catabolism of PAs, and DAP might be important via inducing other stress-related protective mechanisms (Pál et al. 2015).

In conclusion, the present results showed that various mechanisms, including higher proline level, or probably with less importance, increased antioxidant activity could contribute to the elevated heat tolerance of young wheat plants exposed to moderate heat stress. PA levels did not increase after this moderate heat treatment; however, signalling processes related to PA metabolisms or SA-related processes might also contribute to the higher heat tolerance. Most of these processes were mainly temperature dependent, and the differences between these genotypes were less pronounced than the effects of moderate heat stress itself.

Author contribution statement TJ: project supervisor, responsible for experimental design, data collection, interpretation of results, and writer of the manuscript, RK: cultivation of plants, performing proline, and antioxidant analyses, JT: performing gene expression analyses, MP: performing analytical (HPLC) measurements, including salicylic acid and PA determinations, and ÉD: edited the manuscript and provided guidance during experimentation.

Acknowledgements Open access funding provided by MTA Centre for Agricultural Research (MTA ATK). The research is funded by the Széchenyi 2020 programme, the European Regional Development Fund and the Hungarian Government (GINOP-2.3.2-15-2016-00029).

References

Ádám AL, Bestwick CS, Barna B, Mansfield JW (1995) Enzymes regulating the accumulation of active oxygen species during the hypersensitive reaction of bean to *Pseudomonas syringae* pv. *phaseolicola*. Planta 197:240–249

Alcázar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Parrasco C, Tiburcio AF (2010) Polyamines: molecules with regulatory functions in plant abiotic stresstolerance. Planta 231:1237–1249

Bagni N, Tassoni A (2001) Biosynthesis, oxidation and conjugation of aliphatic polyamines in higher plants. Amino Acids 20:301–317

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207

Battisti DS, Naylor RL (2009) Historical warnings of future food insecurity with unprecedented seasonal heat. Science 323:240–244

Camejo D, Martí MDC, Nicolás E, Alarcón JJ, Jiménez A, Sevilla F (2007) Response of superoxide dismutase isoenzymes in tomato plants (*Lycopersicon esculentum*) during thermo-acclimation of the photosynthetic apparatus. Physiol Plant 131:367–377

Chen D, Shao Q, Yin L, Younis A, Zheng B (2019) Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. Front Plant Sci 9:1945

Dat JF, Lopez-Delgado H, Foyer CH, Scott IM (1998) Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol 116:1351–1357

Foley S, Navaratnam S, McGarvey DJ, Land EJ, Truscott G, Rice-Evans CA (1999) Singlet oxygen quenching and the redox properties of hydroxycinnamic acids. Free Rad Biol Med 26:1202–1208

Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int J Mol Sci 14:9643–9684

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012) Role of proline under changing environments. Plant Signal Behav 7:1456–1466

Huang M, Mheni N, Brown-Guedira G, McKendry A, Griffey C, Van Sanford D, Costa J, Sneller C (2018) Genetic analysis of heading date in winter and spring wheat. Euphytica 214:128

Janda T, Szalai G, Tari I, Páldi E (1999) Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. Planta 208:175–180

Janda T, Szalai G, Leskó K, Yordanova R, Apostol A, Popova LP (2007) Factors contributing to enhanced freezing tolerance in wheat during frost hardening in the light. Phytochemistry 68:1674–1682

Janda K, Hideg E, Szalai G, Kovács L, Janda T (2012) Salicylic acid may indirectly influence the photosynthetic electron transport. J Plant Physiol 169:971–978

Khan MIR, Iqbal N, Masood A, Per TS, Khan NA (2013) Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. Plant Signal Behav 8:e26374

Khan SU, Din JU, Qayyum A, Jan NE, Jenks MA (2015a) Heat tolerance indicators in Pakistani wheat (*Triticum aestivum* L.) genotypes. Acta Bot Croat 74:109–121

Khan MI, Fatma M, Per TS, Anjum NA, Khan NA (2015b) Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front Plant Sci 6:462

Kumar RR, Goswami S, Sharma SK, Singh K, Gadpayle KA, Kumar N, Rai GK, Singh M, Rai RD (2012) Protection against heat stress in wheat involves change in cell membrane stability, antioxidant enzymes, osmolyte, H_2O_2 and transcript of heat shock protein. Int J Plant Physiol Biochem 4:83–91

Larkindale J, Knight MR (2002) Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol 128:682–695

Li Q, Wang W, Wang W, Zhang G, Liu Y, Wang Y, Wang W (2018) Wheat F-box protein gene *TaFBA1* is involved in plant tolerance to heat stress. Front Plant Sci 9:521

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408

Majláth I, Szalai G, Soós V, Sebestyén E, Balázs E, Vanková Radomíra, Dobrev Petre I, Tari Irma, Tandori J, Janda T (2012) Effect of light on the gene expression and hormonal status of winter and spring wheat plants during cold hardening. Physiol Plant 145:296–314

Mannervik B, Guthenberg C (1981) Glutathione transferase (Human placenta). Methods Enzymol 77:231–235

Németh M, Janda T, Horváth E, Páldi E, Szalai G (2002) Exogenous salicylic acid increases polyamine content but may decrease drought tolerance in maize. Plant Sci 162:569–574

Pál M, Horváth E, Janda T, Páldi E, Szalai G (2005) Cadmium stimulates the accumulation of salicylic acid and its putative precursors in maize (*Zea mays*) plants. Physiol Plant 125:356–364

Pál M, Szalai G, Janda T (2015) Speculation: polyamines are important in abiotic stress signaling. Plant Sci 237:16–23

Pál M, Majláth I, Németh E, Hamow KA, Szalai G, Rudnóy S, Balassa G, Janda T (2018a) The effects of putrescine are partly overlapping with osmotic stress processes in wheat. Plant Sci 268:67–76

Pál M, Tajti J, Szalai G, Peeva V, Végh B, Janda T (2018b) Interaction of polyamines, abscisic acid and proline under osmotic stress in the leaves of wheat plants. Sci Rep 8(1):12839

Pál M, Ivanovska B, Oláh T, Tajti J, Hamow KA, Szalai G, Khalil R, Vanková R, Dobrev P, Misheva SP, Janda T (2019) Role of polyamines in plant growth regulation of Rht wheat mutants. Plant Physiol Biochem 137:189–202

Pancheva TV, Popova LP (1998) Effect of salicylic acid on the synthesis of ribulose-1,5- biphosphate carboxylase/oxygenase in barley leaves. J Plant Physiol 152:381–386

Paolacci AR, Tanzarella OA, Porceddu E, Ciaffi M (2009) Identification and validation of reference genes for quantitative RT-PCR normalization in wheat. BMC Mol Biol 10:11

Perdomo JA, Conesa M, Medrano H, Ribas-Carbó M, Galmés J (2015) Effects of long-term individual and combined water and temperature stress on the growth of rice, wheat and maize: relationship with morphological and physiological acclimation. Physiol Plant 155:149–165

Proveniers MC, van Zanten M (2013) High temperature acclimation through PIF4 signaling. Trends Plant Sci 18:59–64

Qin D, Wu H, Peng H, Yao Y, Ni Z, Li Z, Zhou C, Sun Q (2008) Heat stress-responsive transcriptome analysis in heat susceptible and tolerant wheat (*Triticum aestivum* L.) by using wheat genome array. BMC Genom 9:432

Shan X, Zhou H, Sang T, Shu S, Sun J, Guo SR (2016) Effects ofexogenous spermidine on carbon and nitrogen metabolism in tomato seedlingsunder high temperature. J Am Soc Hortic Sci 141:381–388

Smith TA (1985) The di- and poly-amine oxidases of higher plants. Biochem Soc Trans 13:319–321

Smith IK, Vierheller TL, Thorne CA (1988) Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). Anal Biochem 175:408–413

Song SQ, Lei YB, Tian XR (2005) Proline metabolism and cross-tolerance to salinity and heat stress in germinating wheat seeds. Russ J Plant Physiol 52:793–800

Szabados L, Savouré A (2010) Proline: a multifunctional amino acid. Trends Plant Sci 15:89–97

Szalai G, Janda K, Darkó É, Janda T, Peeva V, Pál M (2017) Comparative analysis of polyamine metabolism in wheat and maize plants. Plant Physiol Biochem 112:239–250

Uzunova AN, Popova LP (2000) Effect of salicylic acid on leaf anatomy and chloroplast ultrastructure of barley plants. Photosynthetica 38:243–250

Végh B, Marček T, Karsai I, Janda T, Darkó É (2018) Heat acclimation of photosynthesis in wheat genotypes of different origin. S Afr Bot 117:184–192

Wang X, Cai J, Liu F, Jin M, Yu H, Jiang D, Wollenweberc B, Dai T, Cao W (2012) Pre-anthesis high temperature acclimation alleviates the negative effects of post-anthesis heat stress on stem stored carbohydrates remobilization and grain starch accumulation in wheat. J Cereal Sci 55:331–336

Wang J, Wang Y, Gao C, Jiang L, Guo D (2017) PPero, a computational model for plant PTS1 type peroxisomal protein prediction. PLoS ONE 12(1):e0168912

Xiong H, Guo H, Xie Y, Zhao L, Gu J, Zhao S, Li J, Liu L (2017) RNAseq analysis reveals pathways and candidate genes associated with salinity tolerance in a spaceflight-induced wheat mutant. Sci Rep 7(1):2731

Zhao X, Nishimura Y, Fukumoto Y, Li J (2011) Effect of high temperature on active oxygen species, senescence and photosynthetic properties in cucumber leaves. Environ Exp Bot 70:212–216

Zhao XX, Huang LK, Zhang XQ, Li Z, Peng Y (2014) Effects of heat acclimation on photosynthesis, antioxidant enzyme activities, and gene expression in orchardgrass under heat stress. Molecules 19:13564–13576

Zhou H, Xu M, Hou R, Zheng Y, Chi Y, Ouyang Z (2018) Thermal acclimation of photosynthesis to experimental warming is season-dependent for winter wheat (*Triticum aestivum* L.). Env Exp Bot 150:249–259

Zhou R, Kong L, Yu X, Ottosen CO, Zhao T, Jiang F, Wu Z (2019) Oxidative damage and antioxidant mechanism in tomatoes responding to drought and heat stress. Acta Physiol Plant 41:20