

INFLUENCE OF ANTIMYCIN A ON TOTAL PROTEIN CONTENT IN DIFFERENT ORGANS OF WHEAT *TRITICUM AESTIVUM* L. SEEDLINGS UNDER HEAT STRESS

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Abstract

High temperature stress is one of the abiotic stresses that makes wheat crops vulnerable, has a significant impact on crop production worldwide, disrupts cellular homeostasis, and leads to severe retardation of growth and development. This research is aimed assessing the changes in total protein content in etiolated and grown under normal daylight conditions wheat (*Triticum aestivum* L.) seedlings in response to short-term (1 h) and prolonged (24 h) high temperatures (42 °C). In this study, we also examined the role of antimycin A as an activator of the alternative electron transport pathway on changes in total protein content. Results indicated a decrease in total protein content in functionally different organs of etiolated wheat seedlings (4-9%) and those grown under normal daylight conditions (5-11%) following both short-term and prolonged heat stress conditions compared to the control. Antimycin A and high temperature mitigated the decrease in total protein content in functionally different organs of wheat seedlings, suggesting that the activation of alternative electron transport pathway might prevent over-reduction of the electron transport chain, supporting cell homeostasis during abiotic stress conditions.

Keywords: antimycin A, alternative respiration, high temperature, *Triticum aestivum* L.

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INTRODUCTION

High-temperature (HT) stress is one of the primary environmental factors that influences principal physiological and biochemical processes and thus affects the overall plant growth and development, and significantly diminishes plant productivity. Physiological

damages provoked by HT appear at all levels of plant structural organization, including changes in protein structure and enzyme activities as well as alterations in photosynthetic efficiency (Batjuka 2019, Hasanuzzaman et al. 2013). Moreover, it is worth mentioning that HT

negatively affects plant development by inducing misfolding, oxidation, and aggregation of functional and structural proteins, as well as increased fluidity of membrane lipids with loss of membrane integrity (Sachdev et al. 2021). Furthermore, loss of proteins is often associated with heat stress-induced damage and leaf senescence (Anderson & Padhye 2004). Many studies reported that protein degradation was accelerated during heat stress in various plant species (Ngcala et al. 2021, He et al. 2005). It was shown that heat is indirectly associated with enzyme inactivation in chloroplasts and mitochondria, a general inhibition of protein synthesis accompanied by increased protein degradation (Wahid et al. 2007). Furthermore, these damages will surely lead to the inhibition of growth, reduced ion flux, and the generation of reactive oxygen species (ROS) (Wahid et al. 2007). However, plants can maintain growth and reproductive success by sensing alterations in the surrounding environmental conditions and reacting through mechanisms at the cellular, physiological, and developmental levels. Therefore, these response mechanisms enable plants to react rapidly, within hours or days, to extreme climate events that could otherwise cause injury or lethal (Jenks & Hasegawa 2014). Plants increase resistance to heat stress by eliciting specific metabolic adjustments. There may be several mechanisms of adaptation to HT involving the induction and synthesis of molecular chaperones or heat shock proteins, as well as altered protein function (Teery 1980). The processes of recovery and acclimation from HT are essential to ensure plant survival, and the recovery mechanisms, as it is well known, can be independent of the direct heat response mechanisms (Vinocur & Altman 2005).

Plant mitochondria belong to the cell compartments dynamically involved in stress response and stress modulation, which have received more attention from the scientific community in the last decade. The plant mitochondrial electron transport chain (mtETC) has two pathways for the reduction of oxygen:

the cytochrome respiratory pathway (CP) and the nonphosphorylating alternative electron transport pathway (AP), which includes the alternative oxidase (AOX) and some alternative NAD(P)H dehydrogenases. Plant AOX is an interfacial membrane protein oriented toward the matrix side of the inner mitochondrial membrane and couples ubiquinol oxidation to the four-electron reduction of O₂ to H₂O (Vanlerberghe 2013). One proposed function of AP is to prevent the ubiquinone/ubiquinol (UQ) pool from becoming overly reduced, which can result in the production of deleterious quantities of ROS (Zarkovic et al. 2005).

Antimycin A (AA) is a mitochondrial electron transport chain inhibitor that inhibits electron transfer between cytochrome *b* and cytochrome *c*, suppressing the CP and inducing expression of the AP (Djajanegara et al. 2002, Rakhmatullina et al. 2015). However, AA does not bind to the corresponding site of the Cyt b6f complex in chloroplasts. AA inhibits electron transport from recombinant photosynthetic phenotype 1 (PGRL1) to the plastoquinone (PQ) analog (Taira et al. 2013). The activity of AP significantly increased when the terminal part of the main respiratory chain was inhibited by the presence of AA (Rogov et al. 2014).

This study aimed to examine the role of AA as an activator of AP on changes of total protein content in etiolated and grown under normal daylight conditions wheat seedlings exposed to short-term and long-term heat stress.

MATERIALS AND METHODS

Plant materials

Wheat grains (*Triticum aestivum* L., cv. Harmony) were soaked in distilled water and germinated on moistened filter paper in plastic pots (19 × 12 cm) in an environmental climatic chamber (MLR-351H, Versatile Environmental Test Chamber, Sanyo, Japan) in darkness at 26 °C for 24 h. After 24 hours of germination

on wet filter paper at 26 °C, wheat seedlings of equal length were divided into the following control and experimental groups: control group (26 °C), stress conditions (42 °C), and stress conditions (42 °C) in the presence of AA (1 mg/L-1) (Sigma-Aldrich). AA was dissolved in an organic solvent (1 ml ethanol) before being added to the aqueous medium. Control group also received the corresponding amount of solvent to rule out potential solvent effects on protein content. The polyethylene pots were kept in a climate chamber (Sanyo, Versatile Environmental Test Chamber) with the following conditions: day/night temperature, 25±1°C/20±1 °C; relative humidity, 60-70%; 16-h light: 8-h dark photo-cycle; light intensity, 150 µmol m⁻² s⁻¹ for 5 days.

Heat stress treatments

The pots reaching the 5th day of development were allocated to short-term (1 h) and long-term (24 h) high temperature (42°C) treatment as well as in the presence of AA (1 mg l⁻¹) and without AA application in a growth chamber with 70% relative humidity, 16/8 h (light/dark) photoperiod regime and 150 µmol m⁻² s⁻¹ light intensity. Also, control etiolated wheat seedlings and grown under normal daylight conditions grew at 26 °C.

Total protein determination and protein assay

Collected etiolated developing organs (first leaves) and senescent organs (coleoptiles) (0.300 g) were homogenized in a mortar and pestle with ice-cold extraction medium (0,1 M potassium phosphate buffer, pH 7.8, containing 2 mM ethylenediaminetetraacetic acid (EDTA), 8 mM MgCl₂, 4 mM DTT, 0,1% Triton X-100, and 2% soluble polyvinylpyrrolidone (PVP). All operations of enzyme extractions were carried out at 4 °C. The homogeneous suspension was subjected to centrifugation at 12,000 g for 20 min in a refrigerated

centrifuge at 4 °C. After centrifugation, protein concentrations in the supernatant of the enzyme extracts were determined by the “Invitrogen” protocol using the Qubit Quant-iT™ protein assay detection kit and Qubit™ fluorometer (Invitrogen™, USA).

Statistical analysis

The data from all experiments were subjected to one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test using the statistical software Statistica 2010 program, and the means of the treatments were considered to be statistically significant when *P*-level ≤ 0.05. The data from all experiments were the mean of three replicates (*n* = 3), and error bars were expressed as the standard errors (SEs) of the means.

Abbreviations: AA – antimycin A, AOX – alternative oxidase, AP – alternative electron transport pathway, HT – high temperature, mtETC – mitochondrial electron transport chain.

RESULTS AND DISCUSSION

To understand the role of mitochondrial electron transport and AOX in adaptation to thermal stress, we study the role of AA as an activator of AP on changes of total protein content in etiolated and grown under normal daylight conditions wheat seedlings exposed to short-term (1 h) and long-term (24 h) HT (42°C).

The analysis of the obtained results demonstrated that the total protein content was reduced by short-term (4%) and prolonged HT (9%) (Fig. 1) in the first leaf of etiolated wheat seedlings. The results also suggest that the total protein content decreased (*p* < 0.05) in the first leaves of wheat seedlings following short-term (5%) and prolonged (11%) HS grown under normal daylight conditions (Fig. 3). This reduction might be due to

inhibition of protein synthesis at higher temperatures since the injury from high temperatures has often been attributed to the denaturation of proteins, which in general starts with temperatures above 40–50°C (Daniel et al. 1996). The denaturation temperature of plant proteins varies, but according to some sources, it is usually in the range of 70–110°C. Factors like protein type, source, and presence of other ingredients can influence this temperature. Oat proteins are known for their heat stability, with a denaturation temperature around 110°C. For example, some pea proteins may start to denature around 69–77°C, while others, like legumin subunits, may denature and aggregate between 75–85°C. As a confirmation of this assumption, researchers have shown that thermal stress enhances the inhibition of protein synthesis (Duncan and Herschey 1989,

Huang and Xu 2008, Hu et al. 2020). Similarly, a decrease in total protein content was observed in strawberry (*Fragaria x ananassa*) leaves under heat stress (Gulen and Eris 2004). A characteristic feature of the response to heat stress is a reduction in the synthesis of normal proteins and an increase in the synthesis of heat shock proteins (Borovik & Grabelnych 2018), which appear when the temperature increase occurs more gradually to acclimate the plant to high temperatures (Al-Wahaibi 2011). It is also reported that the heat shock proteins play a role in heat tolerance, perhaps by protecting enzymes and nucleic acids from heat denaturation (Wang et al. 2014). In addition, the decline in protein content during HS may be associated with increased proteolytic activity and an increase in free amino acid accumulation in some plant species (He et al. 2005).

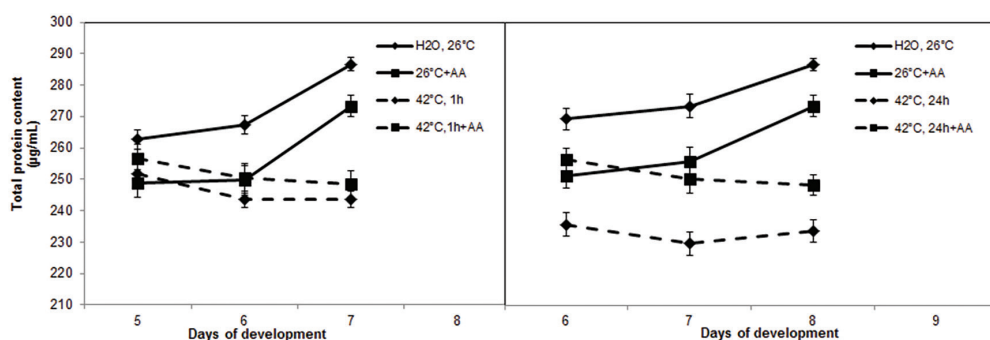


Fig. 1. Effect of short-term (1 h) and long-term (24 h) heat stress and antimycin A on the total protein content on the 5th, 6th and 7th and 8th days of development in etiolated developing organ of *Triticum aestivum* L. Data shown are Mean $\pm$ SE. Each treatment consisted of three replicates.

Unlike the initial leaves, in etiolated coleoptiles and grown under normal daylight, which function comparatively for a short period, protein content decreased ($p < 0.05$) by 3% exposed to AA treatment on the fifth day of development (Fig. 2). The present study demonstrates that the total protein content decreased in senescent organs of etiolated wheat seedlings by short-term and long-term HT (8%) ($p > 0.05$) (Fig. 2). In the present

study, the total protein content was reduced in coleoptiles grown under normal daylight conditions following short-term HT (8%) and long-term HT (11%) ($p < 0.05$) (Fig. 4). Loss of proteins is often associated with heat stress injury and leaf senescence (He et al. 2005). It was shown that total protein concentration decreased significantly in coleoptiles of wheat seedlings under stress conditions (Barassi et al. 1980). The progressive decrease in total

protein content under heat stress could be due to impairment of protein synthesis and/or increased degradation. Consistent with this suggestion, Vanyushin et al. (2004) reported that a second peak of increased $O_2^{\cdot -}$ production observed at the 6th day of wheat seedling development coincided with a decrease in DNA and protein content and apoptotic internucleosomal fragmentation of nuclear DNA in coleoptiles. Shorning et al. (2000) determined that the synthesis of DNA and protein is accompanied by $O_2^{\cdot -}$ production. Similarly, Hameed et al. (2014) also reported

that coleoptile growth was ceased in six-day-old control seedlings, accompanied by intense internucleosomal fragmentation of nuclear DNA associated with apoptotic processes and persistent reorganization in etiolated plants and those growing during normal vegetation. The situation revealed that protein breakdown is one of the most important catabolic processes in senescing leaves, which involves many plastidial and nuclear proteases, as emphasized in previous studies (Diaz-Mendoza et al. 2016, Veliz et al. 2020).

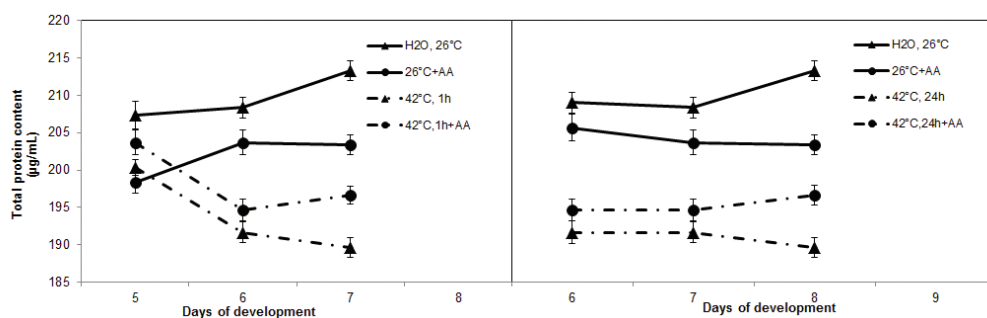


Fig. 2. Effect of short-term (1 h) and long-term (24 h) heat stress and antimycin A on the total protein content on the 5th, 6th and 7th and 8th days of development in etiolated senescent organ of *Triticum aestivum* L. Data shown are Mean $\pm$ SE. Each treatment consisted of three replicates.

Unlike thermal stress alone, the application of AA, which is an activator of the AOX (Wagner, 1997, Geisler et al. 2004, Strodtkötter et al. 2009), mitigated this decrease in total protein content in etiolated developing and senescent organs of wheat seedlings and grown under normal daylight conditions. The results are consistent with those of Zentgraf et al. (2012), who also found that total protein content differed only slightly in AA-treated plants. The results demonstrate that the combined effect of AA and short-term HT did not significantly alter the total protein content in etiolated (2%) ($p < 0.001$) developing organs of wheat seedlings and grown under normal daylight (3%) conditions (Fig. 1). However, the combined effect of AA and long-term HT decreased the total protein content by 6%

in etiolated developing organs ($p < 0.05$) and by 4% grown under normal daylight ($p < 0.05$) (Fig. 3). In this study, we determined that AA treatment decreased the total protein content in etiolated senescent organs (7%) and grown under normal daylight conditions (2%) during prolonged stress conditions in comparison to those in the heat-stressed group (Fig. 2, 4). Geisler et al. (2004) suggest that AA application may slightly increase the protein content in *Solanum tuberosum* L. leaves. The strong activation of the AOX activity might be related indirectly to the low ROS concentration. Consistent with this hypothesis, Jayawardhane et al. (2020) reported that under stress conditions, AOX was important to maintain respiratory carbon flow, preventing the mitochondrial generation of $O_2^{\cdot -}$ and

controlling lipid peroxidation and protein S-nitrosylation. Moreover, it has been noted that the level of total protein can fall rapidly during leaf senescence, which is transported to other actively growing tissues, and this is paralleled by an increase in transcription and activity of several different classes of proteases,

including vacuolar processing enzymes (Araújo et al. 2011). Furthermore, long-term AA treatment, which inhibits complex III of the respiratory chain and also has effects on the cyclic electron flow in the chloroplasts, did not significantly alter senescence in plants (Zentgraf et al. 2022).

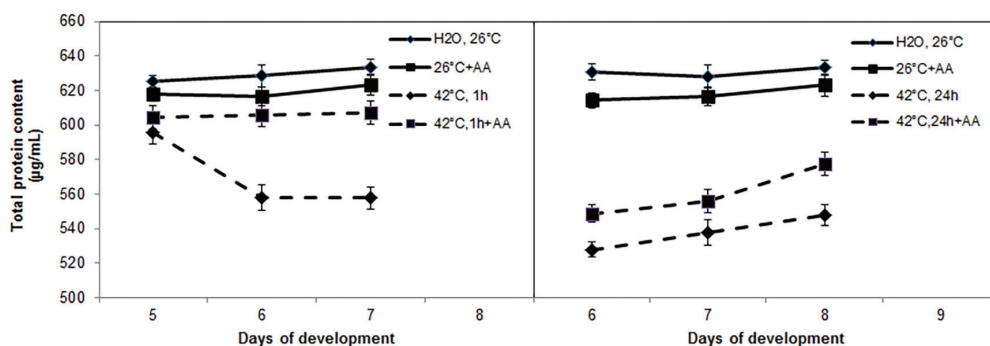


Fig. 3. Effect of short-term (1 h) and long-term (24 h) heat stress and antimycin A on the total protein content on the 5th, 6th and 7th and 8th days of development in developing organ of *Triticum aestivum* L. grown under normal daylight conditions. Data shown are Mean $\pm$ SE. Each treatment consisted of three replicates.

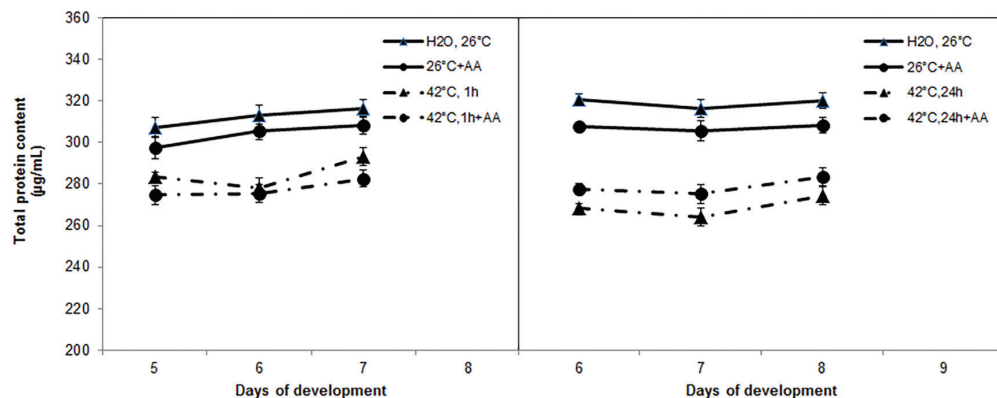


Fig. 4. Effect of short-term (1 h) and long-term (24 h) heat stress and antimycin A on the total protein content on the 5th, 6th and 7th and 8th days of development in senescent organ of *Triticum aestivum* L. grown under normal daylight conditions. Data shown are Mean $\pm$ SE. Each treatment consisted of three replicates.

CONCLUSIONS

The decreased total protein content was determined in functionally different organs

of etiolated and grown under normal daylight conditions wheat seedlings following short-term

and long-term HT. Wheat seedlings exposed to prolonged HT showed a greater reduction of total protein content than plants exposed to short-term HT. The combined effect of AA and HT mitigated the decrease in total protein content in etiolated developing and senescent organs of wheat seedlings and grown under normal daylight conditions, suggesting that the activation of AP might prevent over-reduction of the ETC, supporting cell homeostasis during abiotic stress conditions.

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