

Assessing the effect of antimycin A on morphophysiological parameters in *Triticum aestivum* L. exposed to high temperature

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ABSTRACT

The present study was conducted to investigate the evaluation of antimycin A (AA) as a modulator of the alternative respiratory pathway (AP) on the intensity of growth processes and some physiological parameters of etiolated wheat seedlings, such as maximum length of root, length of first leaf and coleoptile, fresh and dry weights at the whole plant level, leaf area as well as relative water content, saturation water deficit, degree of leaf succulence and sclerophylly. Generally, exposure to heat stress (HS) alone caused a noticeable reduction in root length (59%), leaf length (30%), plant fresh mass (10%), dry mass (14%), and leaf area (25%) which was consistent with the progressive alteration in water relations. Furthermore, the degree of leaf succulence and degree of leaf sclerophylly were severely affected by HS in wheat seedlings. However, the results demonstrated that the application of AA did not induce such a significant reduction in the leaf length (23%), coleoptile length (5%), plant fresh weight (7%), plant dry weight (3%), and leaf area (21%) as well as water relations in etiolated wheat seedlings compared to heat-stressed plants. Taken together, the activation of AP promoted a mitigating of the damaging effect of HS in wheat seedlings supporting growth processes.

Keywords: heat stress, alternative respiratory pathway, antimycin A, *Triticum aestivum* L.

INTRODUCTION

Various environmental stresses exert adverse effects on plant growth and development by inducing many metabolic and physiological processes for adaptation to new conditions. Plants often experience temperature instability in their natural habitat. The ambient temperature is an important factor determining the geographical distribution of plants and their productivity. The viability of plants under conditions of high-temperature stress lies in various reorganizations of metabolic and physiological processes aimed at adapting the plant organism to changing environmental conditions. The main adaptive changes in plants occur at the biochemical and morphological levels.

Resistance to various stress factors becomes an essential characteristic of plants and largely depends on the adaptive mechanisms of the respiratory process

one of the stages of which is the mitochondrial electron transport chain (ETC). During stressful situations, cells use more energy, and the respiration rate increases (Semikhatova, 1995). One has to note that in the case of an elevated respiration rate during abiotic stress conditions, electron transfer reactions can lead either directly or not directly to a significant increased the levels of reactive oxygen species (ROS) causing damage at the cellular and subcellular levels (Tripathy and Oelmüller, 2012).

Respiration is an important parameter in the growth and development of plants. Plants contain a non-phosphorylating cyanide-insensitive alternative electron transport pathway, which localized on the matrix side of the mitochondrial inner membrane, electron transport through which is not coupled with ATP synthesis and energy accumulation (Rogov and Zvyagil'skaya, 2015). The role of the AP unrelated to energy storage, for example in

alleviating or preventing oxidative stress, has attracted the attention of researchers for a few decades (Rogov et al., 2014). AP is believed to be involved in maintaining respiratory carbon metabolism (Florez- Sarasa et al., 2007), redox balance in ETC (Millenaar and Lambers, 2003) reducing the excessive production of ROS due to the rapid electron release to oxygen, and reducing the degree of restoration of the pool of ubiquinones (Maxwell et al., 1999), especially under stress (Rhoads et al., 2006). AP activation contributes to the protection of ETC of chloroplasts from photoinhibition, by dispersing excess reductive equivalents from chloroplasts (Yoshida et al., 2011). This contributes to the implementation of growth and development programs in a constantly changing environment.

AA is an inhibitor of ubiquinol oxidation by the cytochrome bc_1 complex in the mitochondrial low potential chain which prevents the formation of a proton gradient across the inner membrane, thereby prohibiting oxidative phosphorylation (Quinlan et al., 2011; Antal et al., 2013). AA, blocking the cytochrome complex III of the ETC, can lead to changes in the expression of such mitochondrial genes as alternative oxidase (*aox1a*) and rotenone-insensitive NADPH-dehydrogenase (*ndb1*). Previously, it has been shown that the effect of AA at a concentration of 5 μM contributes to an increase in the expression level of the *aox1a* and *ndb1* genes three times, and at a concentration of 15 μM twice compared to the control. Its impact at a concentration of 25 μM leads to complete inhibition of the transcriptional activity of the genes suggesting that this concentration is already toxic to cells (Popov et al., 2011).

Wheat is the most widely grown crop in the world which represents a major resource for food and feed, especially in Europe and North America. It can be grown in temperate zones as well as in tropical and subtropical regions. This distribution implies that wheat is a versatile crop and well adapted to changing environmental conditions. Heat stress represents worldwide spread abiotic stress, significantly limiting yields and agricultural production of many crop plants including wheat. Analysis

of morphological alterations is useful for studying plant adaptations to environmental stress factors. Morphologically the most typical symptom of a heat stress injury to plants is the reduction of growth (Naz et al., 2018). Given the intensive interest in this area of research, it was aimed to investigate the evaluation of AA, as a modulator of AP on the intensity of growth processes and some physiological parameters of etiolated wheat seedlings, such as maximum length of root, length of first leaf and coleoptile, fresh and dry weights at the whole plant level, leaf area as well as relative water content, saturation water deficit, degree of leaf succulence and degree of leaf sclerophylly.

MATERIALS AND METHODS

Plant material and design of the experiment

The object of this study has been selected etiolated seedlings of wheat (*Triticum aestivum* L., cv. Harmony). Cereal seedlings are convenient models for studying plant physiology and molecular biology because their growth and development are synchronous throughout ontogeny (Vanyushin et al., 2004). After 24 hours of germination on moist filter paper at 26°C etiolated wheat seedlings of equal length were divided into the following control and experimental groups: control group (23°C), stressful conditions (42°C, 24 h) and stressful conditions (42°C, 24 h) in the presence of AA (1 mg L⁻¹) (Sigma-Aldrich) and then polyethylene pots transferred in containers (5.5 l) were kept in a climate chamber (Sanyo, Versatile Environmental Test Chamber) under controlled conditions (23/23 °C day/night temperature, relative humidity 80%). Water and reagent solution (AA) used in pots were replaced daily with freshly prepared solutions. The seedlings were subjected to stressful conditions (42°C, 24 h) and stressful conditions (42°C, 24 h) in the presence of AA (1 mg L⁻¹) (Sigma-Aldrich) on the 3rd day of development. After stressful conditions (42°C, 24 h) as well as subjected to stressful conditions (42°C, 24 h) and AA treatment plant material (roots, coleoptiles, and first leaves) was harvested and analyzed for the following parameters.

Morphological parameters

To assess the impact of prolonged HS and the combined effect of AA and long-term HS on some plant growth parameters e.g. maximum length of root, length of first leaf and coleoptile, fresh (FW) and dry (DW) weights at the whole plant level, 4th, 5th, 6th, 7th, and 8th day-olds wheat seedlings were obtained and their growth parameters were measured using a graduated ruler with a precision of 1 mm. The growth parameter values were compared with those of the control groups. Wheat seedlings were weighed to determine their FW and then were dried in a hot air oven at 80°C for 24 h reaching constant weight to determine the dry biomass weight of the same seedlings (Ali et al., 2009). The leaf area measurements were calculated according to the following equation: Leaf area = Length × Breadth × 0.75 (Chaudhary et al. 2012). For determining the degree of succulence and degree of leaf sclerophylly, the following equations were used: Degree of succulence = Water amount/Leaf area; Degree of sclerophylly = Dry mass/Leaf area (Delf, 1912, Witkoswski & Lamont, 1991).

Physiological parameters

The water status of plants was established by measuring relative water contents (RWC) separately in the developing (first leaves) and senescent (coleoptiles) organs of *Triticum aestivum* L. seedlings (Gao, 2000). The water saturation (WSD) in plant tissues was revealed according to Saleh (2013) from the following equation: WSD (%) = 100 - RWC (%).

Data collection and statistical analysis

The one-way analysis of variance (one-factor ANOVA) was used to determine the differences of all obtained data of this research. The results presented as mean values ± standard errors from three independent experiments each of which was made in 20 replicates, and their statistical significance between means was set at $p < 0.05$. The software used to conduct the statistical analyses was the program *Statistica 2010* (Version 13.0).

RESULTS AND DISCUSSION

Analysis of morphological attributes of wheat seedlings

It is well documented by numerous researchers that HS causes various morphological, biochemical, physiological, metabolic, and molecular alterations in plants (Hasanuzzaman et al., 2013; Fedyaeva et al., 2014; Lyububushkina et al., 2015; Grabelnych et al., 2015; Jumiatus et al., 2016). The current results showed that HS significantly declined the length of the first leaf by 30% (Figure 1) and the maximum length of roots by 59% of etiolated wheat seedlings especially at the late stages of seedling development (7th and 8th days) in comparison with control (Figure 2). These results were in agreement with those of many authors (Rodríguez et al., 2005; Hasanuzzaman et al., 2013) who reported that HS causes a loss of cell water content for which the cell size and ultimately the growth is reduced. The inhibition of wheat growth characteristics under HS would also be due to the reduction in net assimilation rate that is also another reason for the reduced relative growth rate under HS which was confirmed in sugarcane sprouts (Wahid et al., 2007). Wheat coleoptiles function for a relatively short period at the early stage of ontogenesis reaching their peak of development on 4th – 5th days and then gradually die (Vanyushin et al., 2004). The data presented revealed that the prolonged HS exposure did not significantly affect ($p > 0.05$) the coleoptile length at the early and late stages of seedlings development (Figure 3). These results were in agreement with those of Momcilovic and Ristic (2007) who reported that the maize coleoptile was heat tolerant at all stages of seedling development. Moreover, it is worth mentioning that in coleoptiles of wheat seedlings at the early stages of development DNA synthesis occurs actively and most likely HS stimulates cell division and can influence growth processes whereas at the late stages of development (after 6th day) the concentration of DNA in cells decreases and the influence of HS practically was noticed (Vanyushin et al., 2004).

The current results showed that fresh and dry weights of etiolated wheat seedlings were reduced by 10% and 14%, respectively, under exposure of HS in

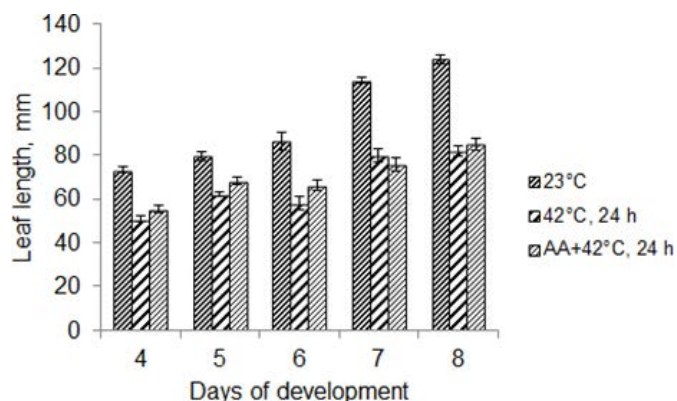


Figure 1. Effect of antimycin A on the length of the first leaves of etiolated wheat seedlings at different stages of development. Data are presented as means \pm SE

comparison with control (Figure 4, 5). These results were in agreement with previous findings of Rodríguez et al. (2015), who reported that high-temperature stress reduced the FW and DW of plants. The inhibition of wheat growth characteristics under HS might be due to alteration in cell division and cell elongation (Fahad et al., 2017). Moreover, a decrease in growth attributes of wheat seedlings under HS could be associated with the production of reactive oxygen species (ROS) which can pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway (Karuppanapandian et al., 2011). The findings of previous research showed an enhanced ROS production in leaves and coleoptiles of etiolated wheat seedlings and seedlings grown under normal daylight regime following short-term and long-term HS (42°C) which was associated with strong oxidative stress leading to membrane biochemical and functional alterations (Batjuka and Škute, 2017; Batjuka and Škute, 2019).

Obtained results indicate that the morphological alterations in *Triticum aestivum* L. after the exogenous AA application after prolonged exposure to HT were significantly affected during developmental stages. The results indicate that the first leaf length of wheat seedlings during early and late-stage under exogenous application of AA was inhibited by 23% and length of coleoptiles by 5% as well as the maximum length of roots by 55% compared to control (Fig. 1, 2, 3). However, unlike the effect of HS alone, AA application improved the morphological

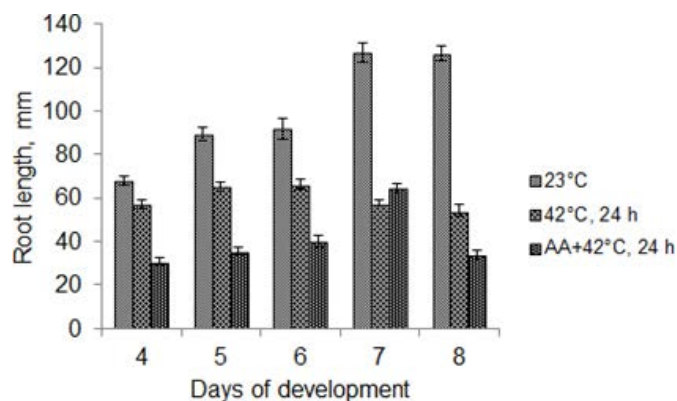


Figure 2. Effect of antimycin A on the length of roots of etiolated wheat seedlings at different stages of development. Data are presented as means \pm SE

attributes of wheat seedlings under HS conditions particularly in first leaves and coleoptiles suggesting that the increased capacity of AP-induced by AA participates in the regulation of many plants physiological processes as well as plant response to oxidative stress conferring tolerance to HT in wheat seedlings. Moreover, the role of AP in the prevention of ROS formation emphasized in previous studies (Amirsadeghi et al., 2006; Gupta et al., 2009; Batjuka, 2019). Consistent with the assumption, Vanlerberghe (2013) has also suggested that AOX activation is the first step in preventing the formation of ROS in plant cells supporting growth processes. At the physiological level, AP cannot prevent the oxidative stress parameters resulting from other metabolic causes except for mitochondrial ETC in the cytosol. Maxwell et al. (1999) determined that the presence of AA, overexpressing *Aox1a* caused induction of growth processes, whereas cells with suppressed levels of AOX died. Sugie et al. (2006) have reported that the growth of *Arabidopsis* was considerably inhibited on the AA-containing medium. Taira et al. (2013) reported that AA inhibited the growth rate of intact roots and leaves in the darkness but in turn root growth was not affected by AA-like compounds in ruptured chloroplasts.

Leaf area is a determinant factor in photosynthesis, radiation interception, biomass accumulation, transpiration, and energy transfer (Akram-Ghaderi and Soltani, 2007) which development is highly sensitive to temperature. The data in Table 1 revealed that HS reduced the leaf area by 25% in comparison with control.

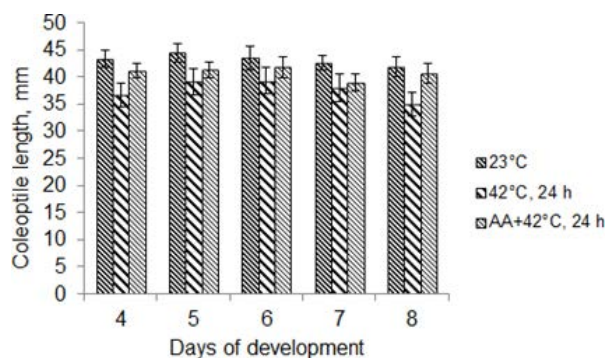


Figure 3. Effect of antimycin A on the length of coleoptiles of etiolated wheat seedlings at different stages of development. Data are presented as means \pm SE

The observed reduction in leaf area of heat-stressed wheat leaves could be attributed to the loss of water content, which causes a reduction in meristematic activity as well as cell elongation, thereby inhibiting leaf expansion. These results were in good agreement with those obtained by Fan et al. (2018). Moreover, wheat seedlings tried to cope with the HS by reducing its leaf area in order to allow the conservation of energy, minimize the deleterious effects of loss of water and activate avoidance and/or tolerance mechanisms. Similarly, the inhibition of the leaf area was alleviated under AA treatment in contrast to HS (Table 1). It is noteworthy that the growth conditions in the presence of AA occur only when the AP is active and is able to support a reduced ATP turnover in plant mitochondria. It is envisioned that AP optimizes the respiratory metabolism during plant growth and development that is important for maintaining metabolic and signaling homeostasis that positively impacts growth, particularly in response to stress factors (Abdrakhimova et al., 2011; Vanlerberghe, 2013).

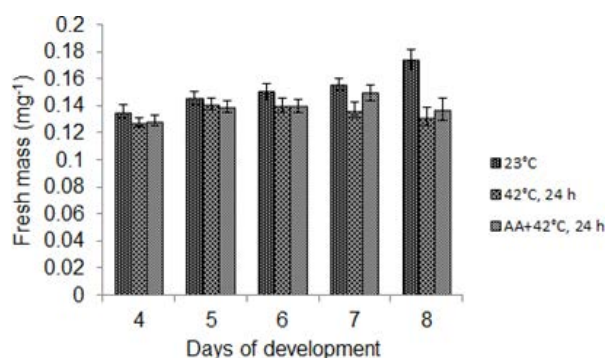


Figure 4. Effect of antimycin A on the fresh mass of etiolated wheat seedlings at different stages of development. Data are presented as means \pm SE

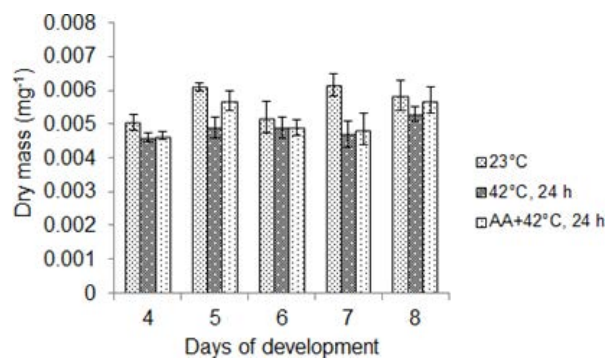


Figure 5. Effect of antimycin A on the dry mass of etiolated wheat seedlings at different stages of development. Data are presented as means \pm SE

Analysis of physiological parameters of wheat seedlings

Leaf relative water content (RWC) is the most prescribed and most efficient parameter of the equilibrium between water absorption and evapotranspiration and is considered the best indicator of the water status of a plant (Farhat et al., 2008). Obtained results indicate that the RWC in the first leaves of wheat seedlings was significantly reduced by 11% with a greater increase in SWD by 29% under long-term exposure to HS depending on the stage of development (Table 1). The results presented above suggest that this may be related not only to the developmental processes of the first leaves but also to the condition of the coleoptile because the primary function of coleoptile is to protect the first leaf from mechanical damage during the germination. However, as distinct from the first leaves coleoptiles more severely (16-19%) lose water content depending on the stage of development (Table 2). Given the fact that coleoptile relatively quickly ages during the development of wheat seedlings (Vanyushin et al., 2004), it is reasonable to hypothesize that HS causes premature senescence. Notably, it was recently observed that RWC in plants was expressively reduced by HS (Kohila and Gomathi, 2018). The progressive decrease of RWC in leaves and coleoptiles of wheat seedlings during abiotic stress conditions is accompanied by a reduction in turgor (Zlatev and Lidon, 2012), suggesting that this could be due to suffering from limited water availability to the cells. Decreasing turgor pressure conceivably leads to a decrease or surcrease of the growth process by decreasing cell extensibility and cell expansion (Gall et al., 2015).

Table 1. Effect of antimycin A on the leaf area, relative water content (RWC), saturation water deficit (SWD), leaf succulence and leaf sclerophylly in the etiolated developing organs of wheat seedlings at different stages of development. Data are presented as means \pm SE

Sample	Leaf area (cm ²)	RWC (%)	SWD (%)	Leaf succulence (mg cm ⁻²)	Leaf sclerophylly
23°C, 4 th day	113.5 \pm 1.3	83.6 \pm 3.7	17.4 \pm 2.4	0.16 \pm 0.02	3.32 \pm 1.1
23°C, 5 th day	195.0 \pm 1.9	87.9 \pm 1.8	12.0 \pm 1.8	0.14 \pm 0.02	2.91 \pm 0.2
23°C, 6 th day	218.3 \pm 4.7	84.2 \pm 2.6	19.2 \pm 0.5	0.09 \pm 0.01	1.78 \pm 0.1
23°C, 7 th day	331.0 \pm 2.7	86.1 \pm 2.9	16.8 \pm 0.6	0.07 \pm 0.01	2.12 \pm 0.0
23°C, 8 th day	365.0 \pm 2.7	82.6 \pm 2.4	14.1 \pm 1.0	0.05 \pm 0.00	1.65 \pm 0.3
42°C, 4 th day	80.5 \pm 1.8	70.4 \pm 3.1	21.4 \pm 2.7	0.14 \pm 0.01	5.84 \pm 1.2
42°C, 5 th day	95.5 \pm 2.2	74.1 \pm 3.1	21.7 \pm 2.5	0.09 \pm 0.01	6.21 \pm 0.8
42°C, 6 th day	168.8 \pm 4.5	75.2 \pm 4.1	19.6 \pm 3.1	0.06 \pm 0.02	3.68 \pm 0.3
42°C, 7 th day	306.3 \pm 2.5	78.3 \pm 2.5	25.9 \pm 3.1	0.05 \pm 0.01	5.79 \pm 0.1
42°C, 8 th day	318.8 \pm 3.8	78.6 \pm 2.7	24.8 \pm 4.1	0.03 \pm 0.00	3.47 \pm 0.2
AA+42°C, 4 th day	85.5 \pm 1.7	77.0 \pm 2.6	24.7 \pm 2.0	0.16 \pm 0.02	5.45 \pm 0.3
AA+42°C, 5 th day	102.0 \pm 4.6	75.8 \pm 1.9	14.2 \pm 1.9	0.12 \pm 0.01	4.25 \pm 0.6
AA+42°C, 6 th day	169.5 \pm 1.9	78.7 \pm 1.5	15.3 \pm 1.5	0.08 \pm 0.01	3.15 \pm 0.4
AA+42°C, 7 th day	310.0 \pm 3.3	81.0 \pm 2.9	19.0 \pm 2.9	0.05 \pm 0.01	2.23 \pm 0.2
AA+42°C, 8 th day	327.5 \pm 3.3	83.5 \pm 3.0	16.5 \pm 3.0	0.04 \pm 0.00	1.71 \pm 0.3

Table 2. Effect of antimycin A on the relative water content (RWC) and saturation water deficit (SWD) in the etiolated senescent organs of wheat seedlings at different stages of development. Data are presented as means \pm SE

Sample	RWC (%)	SWD (%)
23°C, 4 th day	93.2 \pm 1.3	14.7 \pm 2.8
23°C, 5 th day	95.7 \pm 2.5	16.3 \pm 1.9
23°C, 6 th day	93.6 \pm 2.3	15.2 \pm 1.7
23°C, 7 th day	98.2 \pm 1.8	15.9 \pm 0.6
23°C, 8 th day	93.6 \pm 1.2	16.4 \pm 1.5
42°C, 4 th day	75.6 \pm 2.1	16.5 \pm 2.9
42°C, 5 th day	78.3 \pm 2.5	15.2 \pm 2.9
42°C, 6 th day	78.4 \pm 2.8	16.2 \pm 2.2
42°C, 7 th day	79.1 \pm 2.8	15.3 \pm 2.8
42°C, 8 th day	76.2 \pm 2.9	15.7 \pm 4.1
AA+42°C, 4 th day	86.4 \pm 2.6	16.6 \pm 2.3
AA+42°C, 5 th day	89.2 \pm 2.8	16.6 \pm 1.9
AA+42°C, 6 th day	85.0 \pm 2.5	20.5 \pm 1.8
AA+42°C, 7 th day	88.0 \pm 2.3	22.6 \pm 2.3
AA+42°C, 8 th day	85.5 \pm 3.3	21.4 \pm 2.6

Moreover, suppression in RWC under stress conditions might be associated with a reduction of water uptake characterized by the injury to the root system (Garg and Singla, 2009). Another interesting observation from this study is that HS induced a significant reduction (29%) in the degree of leaf succulence although the degree of sclerophylly was significantly increased (53%) (Table 1). These observations are in agreement with previously proposed studies describing a decrease in the degree of leaf succulence in stressed leaves (Welch and Rieseberg, 2002). Taken together, it has been established that this could be affected by the water deficit of the growing leaves, which pointed by less RWC and more SWD as well as less succulence and more sclerophylly.

The application of AA mitigates the effect of HS by maximizing RWC by 5% and minimizing SWD by 29% in the first leaves of wheat seedlings compared to HS alone (Table 1). The results also indicate that exogenous application of AA increased RWC by 11% and SWD by 18% in the coleoptiles of wheat seedlings compared to

HS alone (Table 2). In the present study, when compared to the HS alone, AA treatment markedly increased (17%) the degree of leaf succulence and reduced (33%) the degree of leaf sclerophylly. It is supposed that this enhancement in the developing and senescent organs of wheat seedlings could result from the activation of AP that is efficient in reducing the adverse effects of HS maintaining the relatively high water content necessary for growth and cellular functions and perhaps delays the senescence of whole coleoptiles.

CONCLUSIONS

In summary, the present study indicated that prolonged HS adversely influenced the growth and physiological parameters of etiolated wheat seedlings, such as maximum length of roots, length of first leaf and coleoptile, fresh and dry weights at the whole plant level, leaf area as well as relative water content, saturation water deficit, degree of succulence and degree of sclerophylly which may be attributed due to an enhanced level of oxidative stress. However, the highest stress resistance was determined in wheat seedlings grown under the combined effect of AA and prolonged HS.

REFERENCES

- Abdrakhimova, Y.R., Andreev, I.M., Shugaev, A.G. (2011) Involvement of the energy-dissipating systems in modulating the energetic efficiency of respiration in mitochondria from etiolated winter wheat seedlings. *Russian Journal of Plant Physiology*, 58, 567-574. DOI: <https://doi.org/10.1134/S1021443711040029>
- Akram-Ghaderi, F., Soltani, A. (2007) Leaf area relationships to plant vegetative characteristics in cotton (*Gossypium hirsutum* L.) grown in a temperature sub-humid environment. *International Journal of Plant Production*, 1, 63-71. DOI: <https://doi.org/10.22069/IJPP.2012.526>
- Ali, M.A., Abbas, A., Niaz, S., Zulkiffal, M., Ali, S. (2009) Morphophysiological criteria for drought tolerance in sorghum (*Sorghum Bicolor*) at seedling and post-anthesis stages. *International Journal of Agriculture and Biology*, 11, 674-680.
- Amirsadeghi, S., Robson, C.A., McDonald, A.E., Vanlerberghe, G.C. (2006) Changes in plant mitochondrial electron transport alter cellular levels of reactive oxygen species and susceptibility to cell death signaling molecules. *Plant and Cell Physiology*, 47, 1509-1519. DOI: <https://doi.org/10.1093/pcp/pcl016>
- Antal, T.K., Kukarskikh, G.P., Bulychev, A.A., Tyystjärvi, E., Krendeleva, T. (2013) Antimycin A effect on the electron transport in chloroplasts of two *Chlamydomonas reinhardtii* strains. *Planta* 237, 1241-1250. DOI: <https://doi.org/10.1007/s00425-013-1843-y>
- Batjuka, A. (2019) The impact of antimycin A on some oxidative processes and antioxidant status in the leaves of wheat seedlings (*Triticum aestivum* L.) in response to high temperature. *Agrochimica* 13, 109-119. DOI: <https://doi.org/10.12871/00021857201912>
- Batjuka, A., Škute, N. (2019) Evaluation of superoxide anion level and membrane permeability in the functionally different organs of *Triticum aestivum* L. exposed to high temperature and antimycin A. *Current Science*, 117, 440-447. DOI: <https://doi.org/10.18520/cs/v117/i3/440-447>
- Batjuka, A., Škute, N. (2017) The effect of antimycin A on the intensity of oxidative stress, the level of lipid peroxidation and antioxidant enzyme activities in different organs of wheat (*Triticum aestivum* L.) seedlings subjected to high temperature. *Archives of Biological Sciences*, 69, 743-752. DOI: <https://doi.org/10.2298/ABS160706134B>
- Delf, E.M. (1912) Transpiration in succulent plants. *Annals of Botany*, 26, 409-440. DOI: <https://doi.org/10.1093/oxfordjournals.aob.a089398>
- Fahad, S., Bajwa, A.A., Nazir, U., Anjum, S.A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M.Z., Alharby, H., Wu, C., Wang, D., Huang, J. (2017) Crop production under drought and heat stress: plant responses and management options. *Frontiers in Plant Science*, 8, 1-16. DOI: <https://doi.org/10.3389/fpls.2017.01147>
- Fan, Y., Ma, C., Huang, Z., Abid, M., Jiang, S., Dai, T., Zhang, W., Ma, S., Jiang, D., Han, X. (2018) Heat priming during early reproductive stages enhances thermo-tolerance to post-anthesis heat stress via improving photosynthesis and plant productivity in winter wheat (*Triticum aestivum* L.). *Frontiers in Plant Science*, 9, 1-17. DOI: <https://doi.org/10.3389/fpls.2018.00805>
- Farhat, J., Shahbaz, M., Ashraf, M. (2008) Discriminating some prospective cultivars of maize (*Zea mays* L.) for drought tolerance using gas exchange characteristics and proline contents as physiological markers. *Pakistan Journal of Botany*, 40, 2329-2343.
- Fedyayeva, A.V., Stepanov, A.V., Lyubushkina, I.V., Pobezhimova, T.P., Rikhvanov, E.G. (2014) Heat shock induces production of reactive oxygen species and increases inner mitochondrial membrane potential in winter wheat cells. *Biochemistry (Moscow) Supplement Series A Membrane and Cell Biology*, 79, 1202-1210. DOI: <https://doi.org/10.1134/S0006297914110078>
- Florez-Sarasa, I.D., Bouma, T.J., Medrano, H., Azcon-Bieto, J., Ribas-Carbó, M. (2007) Contribution of the cytochrome and alternative pathways to growth respiration and maintenance respiration in *Arabidopsis thaliana*. *Physiologia Plantarum*, 129, 143-151. DOI: <https://doi.org/10.1111/j.1399-3054.2006.00796.x>
- Gall, H., Philippe, F., Domon, J.M., Gillet, F., Pelloux, J., Rayon, C. (2015) Cell wall metabolism in response to abiotic stress. *Plants* 4, 112-166. DOI: <https://doi.org/10.3390/plants4010112>
- Gao, F. (2000) *Experimental technology in plant physiology*. World Books Publishing Company, China.
- Garg, N., Singla, R. (2009) Variability in the response of chickpea cultivars to short-term salinity, in terms of water retention capacity, membrane permeability and osmo-protection. *Turkish Journal of Agriculture and Forestry*, 33, 57-63.
- Grabelnich, O.I., Pobezhimova, T.P., Koroleva, N.A., Fedoseeva, I.V., Stepanov, A.V., Lyubushkina, I.V., Zhivetev, M.A., Graskova, I.A., Voinikov, V.K. (2015) Temperature stress and consequences of its influence on functional activity of mitochondria in maize etiolated seedlings. *Journal of Stress Physiology & Biochemistry*, 11, 82-93.

- Gupta, K.J., Zabalza, A., Van Dongen, J.T. (2009) Regulation of respiration when the oxygen availability changes. *Physiologia Plantarum*, 137, 383-391. DOI: <https://doi.org/10.1111/j.1399-3054.2009.01253.x>
- Hasanuzzaman, M., Nahar, K., Alam, M., Roychowdhury, R., Fujita, M. (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences*, 14, 9643-9684. DOI: <https://doi.org/10.3390/ijms14059643>
- Jumiatus, Junaedi A., Lubis, I., Chozin, M.A., Miyazaki, A. (2016) Morphological, physiological and yield responses of some rice varieties (*Oryza sativa* L.) as exposed under high temperature in Indonesia. *American Journal of Plant Physiology*, 11, 33-41. DOI: <https://doi.org/10.3923/ajpp.2016.33.41>
- Karuppanapandian, T., Moon, J.C., Kim, C., Manoharan, K., Kim, W. (2011) Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. *Australian Journal of Crop Science*, 5, 709-725.
- Kohila, S., Gomathi, R. (2018) Adaptive physiological and biochemical response of sugarcane genotypes to high-temperature stress. *Indian Journal of Plant Physiology*, 23, 245-260. DOI: <https://doi.org/10.1007/s40502-018-0363-y>
- Luybushkina, I.V., Fedyeva, A.V., Stepanov, A.V., Pobezhimova, T.P. (2015) Changes of respiration activities in cells of winter wheat and sugar cane suspension cultures during programmed cell death process. *Journal of Stress Physiology & Biochemistry*, 11, 71-81.
- Maxwell, D.P., McIntosh, L. (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proceedings of the National Academy of Sciences of the United States of America*, 96(14), 8271-8276. DOI: <https://doi.org/10.1073/pnas.96.14.8271>
- Millenaar, A.H., Lambers, H. (2003) The alternative oxidase: in vivo regulation and function. *Plant Biology*, 5, 2-15. DOI: <https://doi.org/10.1055/s-2003-37974>
- Momcilovic, I., Ristic, Z. (2007) Expression of chloroplast protein synthesis elongation factor, EF-Tu, in two lines of maize with contrasting tolerance to heat stress during early stages of plant development. *Journal of Plant Physiology*, 164, 90-99. DOI: <https://doi.org/10.1016/j.jplph.2006.01.010>
- Naz, N., Durrani, F., Shah, Z., Khan, NA, Ullah, I. (2018) Influence of heat stress on growth and physiological activities of potato (*Solanum tuberosum* L.). *PHYTON*, 87, 225-230.
- Popov, V.N., Eprintsev, A.T., Maltseva, E.V. (2011) Activation of genes encoding mitochondrial proteins involved in alternative and uncoupled respiration of tomato plants treated with low temperature and reactive oxygen species. *Russian Journal of Plant Physiology*, 58, 914-920. DOI: <https://doi.org/10.1134/S1021443711040091>
- Quarrie, S.A. Jones, H.G. (1979) Genotype variation in leaf water potential, stomatal conductance and abscisic acid concentration in spring wheat subjected to artificial drought stress. *Annals of Botany*, 44, 323-332. DOI: <https://doi.org/10.1093/oxfordjournals.aob.a085736>
- Quinlan, C.L., Gerencser, A.A., Treberg, J.R., Brand, M.D. (2011) The mechanism of superoxide production by the antimycin-inhibited mitochondrial Q-cycle. *The Journal of Biological Chemistry*, 286, 31361-31372. DOI: <https://doi.org/10.1074/jbc.M111.267898>
- Rhoads, D.M., Umbach, A.L., Subbaiah, C.C., Siedow, J.N. (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. *Plant Physiology*, 141, 357-366. DOI: <https://doi.org/10.1104/pp.106.079129>
- Rodríguez, M., Canales, E., Borrás-Hidalgo, O. (2005) Molecular aspects of abiotic stress in plants. *Applied biochemistry and Biotechnology*, 22, 1-10.
- Rodríguez, V.M., Soengas, P., Alonso-Villaverde, V., Sotelo, T., Cartea, M.E., Velasco, P. (2015) Effect of temperature stress on the early vegetative development of *Brassica oleracea* L. *BMC Plant Biology*, 15, 1-9. DOI: <https://doi.org/10.1186/s12870-015-0535-0>
- Rogov, A.G., Sukhanova, E.I., Uralskaya, L.A., Aliverdieva, D.A., Zvyagil'skaya, R.A. (2014) Alternative oxidase: distribution, induction, properties, structure, regulation, and functions. *Biochemistry (Moscow)*, 79, 1615-1634. DOI: <https://doi.org/10.1134/S0006297914130112>
- Rogov, A.G., Zvyagil'skaya, R.A. (2015) Physiological role of alternative oxidase (from yeasts to plants). *Biochemistry (Moscow)*, 80, 400-407. DOI: <https://doi.org/10.1134/S0006297915040021>
- Semikhatova, O.A. (1995) Maintenance respiration and the cost of plant adaptation. *Russian Journal of Plant Physiology*, 42, 277-284.
- Sugie, A., Naydenov, N., Mizuno, N., Nakamura, C., Takumi, S. (2006) Overexpression of wheat alternative oxidase gene *Waox1a* alters respiration capacity and response to reactive oxygen species under low temperature in transgenic Arabidopsis. *Genes and Genetic Systems*, 81, 349-354. DOI: <https://doi.org/10.1266/ggs.81.349>
- Taira, Y., Okegawa, Y., Sugimoto, K., Abe, M., Miyoshi, H., Shikanai, T. (2013) Antimycin A-like molecules inhibit cyclic electron transport around photosystem I in ruptured chloroplasts. *FEBS Open Bio* 3, 406-410. DOI: <https://doi.org/10.1016/j.fob.2013.09.007>
- Tripathy, B.C., Oelmüller, R. (2012) Reactive oxygen species generation and signalling in plants. *Plant Signaling & Behavior*, 7, 1621-1633. DOI: <https://doi.org/10.4161/psb.22455>
- Vanlerberghe, G.C. (2013) Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. *International Journal of Molecular Sciences*, 14, 6805-6847. DOI: <https://doi.org/10.3390/ijms14046805>
- Vanyushin, B.F., Bakeeva, L.E., Zamyatnina, V.A., Aleksandrushkina, N.I., 2004. Apoptosis in plants: specific features of plant apoptotic cells and effect of various factors and agents. *International Review of Cytology*, 233, 135-179. DOI: [https://doi.org/10.1016/S0074-7696\(04\)33004-4](https://doi.org/10.1016/S0074-7696(04)33004-4)
- Wahid, A. (2007) Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *Journal of Plant Research*, 120, 219-228. DOI: <https://doi.org/10.1007/s10265-006-0040-5>
- Weatherly, P.E., and Barrs, C. (1962) A re-examination of relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences*, 15, 413-428. DOI: <https://doi.org/10.1071/BI9620413>
- Welch, M.E., Rieseberg, L.H. (2002) Habitat divergence between a homoploid hybrid sunflower species, *Helianthus paradoxus* (Asteraceae), and its progenitors. *American Journal of Botany*, 89, 472-478. DOI: <https://doi.org/10.3732/ajb.89.3.472>
- Witkowski, E.T.F., and Lamont, B.B. (1991) Leaf specific mass confounds leaf density and hickness. *Oecologia*, 84, 362-370. DOI: <https://doi.org/10.1007/BF00317710>
- Yoshida, K., Watanabe, C.K., Terashima, I., Noguchi, K. (2011) Physiological impact of mitochondrial alternative oxidase on photosynthesis and growth in Arabidopsis thaliana. *Plant, Cell & Environment*, 34, 1890-1899. DOI: <https://doi.org/10.1111/j.1365-3040.2011.02384.x>
- Zlatev, Z., Lidon, F.C. (2012) An overview on drought induced changes in plant growth, water relations and photosynthesis. *Emirates Journal of Food and Agriculture*, 24, 57-72. DOI: <https://doi.org/10.9755/ejfa.v24>