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COMPARISON OF THE EFFECT OF HIGH LIGHT INTENSITY ON THE RESPONSE OF *SOLANUM LYCOPERSICUM* AND *ARABIDOPSIS THALIANA* TO LOW TEMPERATURE

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Abstract

This study compares the effect of light intensity on the responses of two plants with different cold tolerance – tomato (*Solanum lycopersicum* L.) and on model plant Arabidopsis (*Arabidopsis thaliana* Col 0) to several days' treatment by low temperature and after recovery at control conditions. Net photosynthetic rate was evaluated by oxygen evolution from leaf discs. The photosynthetic performance was assessed by the maximal quantum efficiency of photosystem II and the degree of non-photochemical and non-regulated quenching. Malondialdehyde accumulation was used as a stress indicator and synthesis and accumulation of anthocyanins as an indicator for activation of defense mechanism. The results demonstrated that in Arabidopsis plants the high light treatment at control and low temperature increased non-regulated quenching but, in tomato plants the balance between both quenching mechanisms was maintained.

Key words: anthocyanins, malondialdehyde, oxygen evolution, photosystem II, non-photochemical quenching

Abbreviations: ΦNO – nonregulated non-photochemical quenching, Fv/Fm – maximal quantum efficiency of PSII, MDA – malondialdehyde, NPQ – non-photochemical quenching, PSII – photosystem II, ROS – reactive oxygen species

Introduction. One of the most significant environmental constrains on plant physiology is exposure to cold temperatures which significantly affects productivity, photosynthesis, and gene expression [1, 2]. According to research on barley

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and *Arabidopsis thaliana* low temperatures reduce the possibility of state 1-state 2 transition, which balance the absorbed light energy between the two photosystems in a short time by relocating light-harvesting complex II proteins [3, 4]. Light conditions have a considerable impact on the degree of cold-induced damage [5]. To cope with combined stress, plants have evolved defense processes, as the production of protective compounds, changes of protein and lipid composition, activation of ROS-scavenging enzymes [6]. Under combined stress PSII was either overexcited or underexcited due to an imbalance between photochemical processes and the metabolic utilization of excitation energy resulting in production of ROS [7]. This causes photooxidative damage and photoinhibition of PSII of chilling-sensitive plants in particular [8, 9]. Being a plant from tropical regions, tomato is relatively sensitive to exposure to suboptimal temperatures [10]. The exposure of tomato to low temperature at optimal light intensity resulted in alterations of pigment content and effectiveness of oxygen evolution [11]. Arabidopsis was classified as a cold-tolerant species based on tests for low temperature resistance and for low temperature responsive genes [12].

Although the damaging effects of low temperature on photosynthesis are well documented, experimental data on the effects of high light intensity during cold stress and its impact during recovery are scarce [11, 12]. In the current study we assessed and compared how tomato and Arabidopsis responded to exposure to low, non freezing temperatures and the impact of high light intensity on both plants' acclimation. The change in pigment content and oxygen evolution were used to assess the photosynthetic performance. PAM fluorescence analysis was used to determine photosystem II activity, NPQ and ΦNO quenching. MDA accumulation served as stress indicator. The accumulation of anthocyanins as protective compounds was monitored.

Materials and methods. ***Plant material.*** Tomato plants (*Solanum lycopersicum* cv. UC 80) were grown as described in [11]. At the stage of 3rd leaf the plants were treated for 6 days at four different conditions – NT–NL (22/20 °C and 250 µmol m^{-2} s^{-1}), LT–NL (12/10 °C and 250 µmol m^{-2} s^{-1}), LT–HL (12/10 °C and 800 µmol m^{-2} s^{-1}) and NT–HL (22/20 °C and 800 µmol m^{-2} s^{-1}). After treatment the plants were returned to normal conditions (NT–NL) and allowed to recover for 5 days, noted hereafter (5 days R). *Arabidopsis thaliana* Col-0 plants were grown as described in [13]. Thirty-eight days old plants were divided into four groups and were treated for 6 days under different regimes as follows: NT–NL (21/18 °C and 150 µmol m^{-2} s^{-1}), LT–NL (12/10 °C and 150 µmol m^{-2} s^{-1}), LT–HL (12/10 °C and 500 µmol m^{-2} s^{-1}) and NT–HL (21/18 °C and 500 µmol m^{-2} s^{-1}). Our preliminary results and literature data indicate that the conditions for normal growth of Arabidopsis and tomato differ little in terms of temperature but quite a bit in terms of light intensity. The temperature and light intensity for stress treatments were chosen to be three times higher than those for growth of the

respective plant. After treatment the plants were returned to control conditions and allowed to recover for 5 days.

Determination of pigment content. Pigment content of leaves (Chl $(a+b)$ and carotenoids was estimated in 80% acetone using the equations of LICHTENTHALER [14].

Photosynthetic O_2 evolution from leaf discs. Net photosynthesis of control and treated tomato and Arabidopsis plants was determined by CO_2-saturated O_2 evolution of leaf discs using a Clark-type O_2 electrode (model DW1, Hansatech Instruments, UK). Measurements were performed using 8 leaf discs with a total area of 10 cm^2 that were dark-adapted for 5 min and in a saturating atmosphere of CO_2, provided by 200 µl 1M $NaHCO_3$, at temperature 22 °C and at saturating light intensity of 800 µmol m^{-2} s^{-1}. At least 4 measurements were performed for every time point.

PAM chlorophyll a fluorescence and determination of NPQ and ΦNO. In vivo chlorophyll *a* fluorescence was measured by using PAM fluorometer (H. Walz, Germany, model PAM 101–103) using detached dark adapted (15 min) leaves and calculation of NPQ, Fv/Fm and ΦNO were performed as described in [15].

Determination of MDA. The malondialdehyde (MDA) content in tomato and Arabidopsis leaves was determined by the thiobarbituric acid method as described in [16].

Determination of anthocyanin content. The content of accumulated anthocyanins was estimated by the formula: $A_{Anth} = A_{535} - 0.24 \times A_{653}$ as described in [16].

Statistics. The results in all figures represent the mean values ± SE, calculated from three or four independent experiments with 4 parallel samples. Data were statistically compared by Fisher's Least Significant Difference test at $p \leq 0.05$ of ANOVA analysis. Statistically significant differences between values were indicated with different letters.

Results. ***Changes of pigment content.*** After 2 days under LT–NL conditions no significant changes in Chl and Car content of both plants were detected. Noticeable difference was observed after 6 days treatment by LT–NL and LT–HL. In tomato plants treated by LT–NL the content of Chl $(a+b)$ decreased by about 25%, but in Arabidopsis plants a significant reduction (about 35%) of Chl $(a+b)$ was detected on the 6th day at LT–HL (Fig. 1). Similar behaviour was observed for Car content in both plants. During recovery period both plants successfully restored pigment content to the level of control.

Net photosynthesis and Fv/Fm. Exposure of tomato and Arabidopsis plants to LT–NL and LT–HL affected O_2 evolution in different manner and in a time dependent way (Fig 2A, B). LT–NL treatment reduced photosynthesis in tomato and this reduction depended on the days of treatment – by about 25% on the 2nd day and by 40% on the 6th day. After 5 days at control conditions the

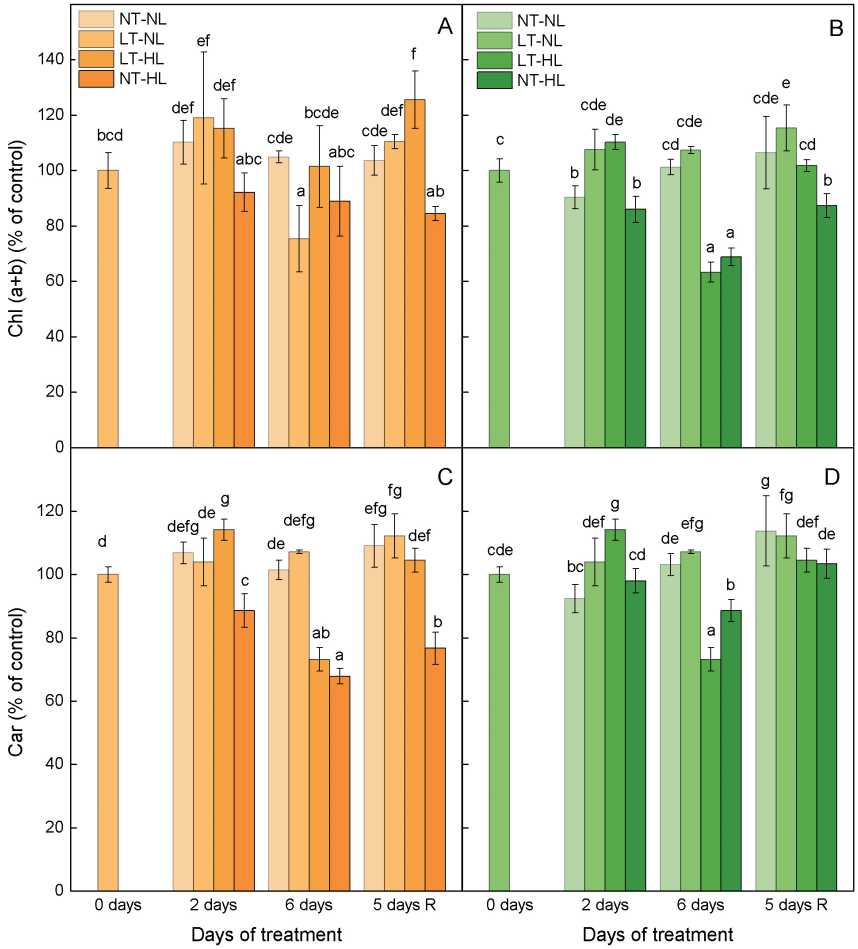


Fig. 1. Changes in the content of chlorophylls (Chl $(a+b)$) and total carotenoids (Car) in tomato (A and C) and Arabidopsis (B and D) plants after treatment by LT–NL, LT–HL, and NT–HL for 6 days and after 5 days recovery (5 days R). For tomato 100% correspond to 1.761 mg g^{-1} FW for Chl $(a+b)$ and 0.579 mg g^{-1} FW for Car; for Arabidopsis 100% correspond to 0.989 mg g^{-1} FW for Chl $(a+b)$ and 0.309 mg g^{-1} FW for Car

photosynthetic activity recovered nearly to the initial value. O_2 evolution rate was almost not affected in Arabidopsis by LT–NL but decreased after treatment by LT–HL – by about 25% on the 2nd and 6th day. Comparing the effect of high light intensity, it should be mentioned that 6 days treatment by NT–HL resulted in significant reduction of O_2 evolution in Arabidopsis. During recovery period no restoration of O_2 evolution was detected for LT–HL treated Arabidopsis and the restoration for LT–HL treated tomato was not statistically significant. Fv/Fm behaved similarly during the first two days of treatment of tomato and Arabidopsis

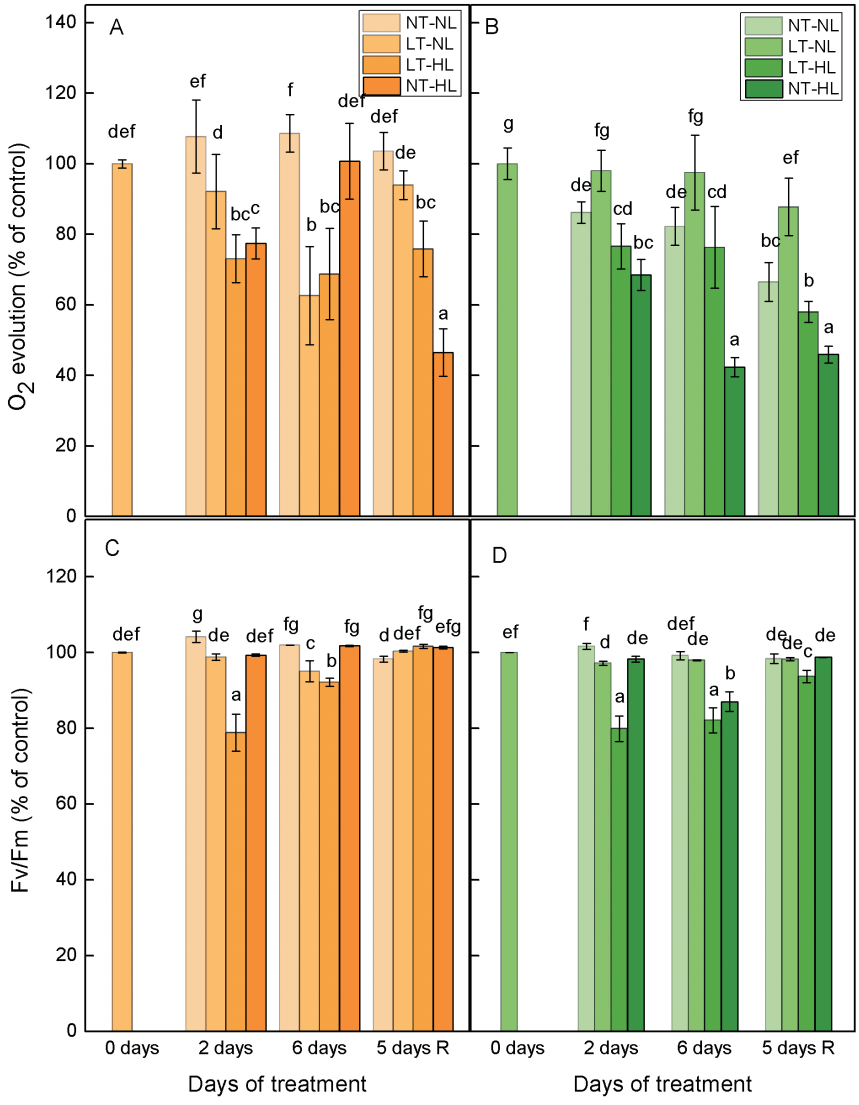


Fig. 2. Net photosynthesis measured as CO_2-saturated O_2 evolution of leaf discs from tomato (A) and Arabidopsis (B) plants and changes of maximal quantum efficiency of PSII (Fv/Fm) in tomato (C) and Arabidopsis (D) after different treatments. 100% of O_2 evolution corresponds to 12.279 µmol O_2 m^{-2} s^{-1} (tomato) and to 9.315 µmol O_2 m^{-2} s^{-1} (Arabidopsis). 100% of Fv/Fm corresponds to 0.82 for tomato and 0.85 for Arabidopsis

(a reduction of about 20% in LT–HL was found for both plants), but there was a difference on the 6th day: in Arabidopsis the ratio Fv/Fm was more significantly affected by LT–HL and NT–HL treatment (Fig. 2C, D). The Fv/Fm ratio was restored to the control values after 5 days at control conditions.

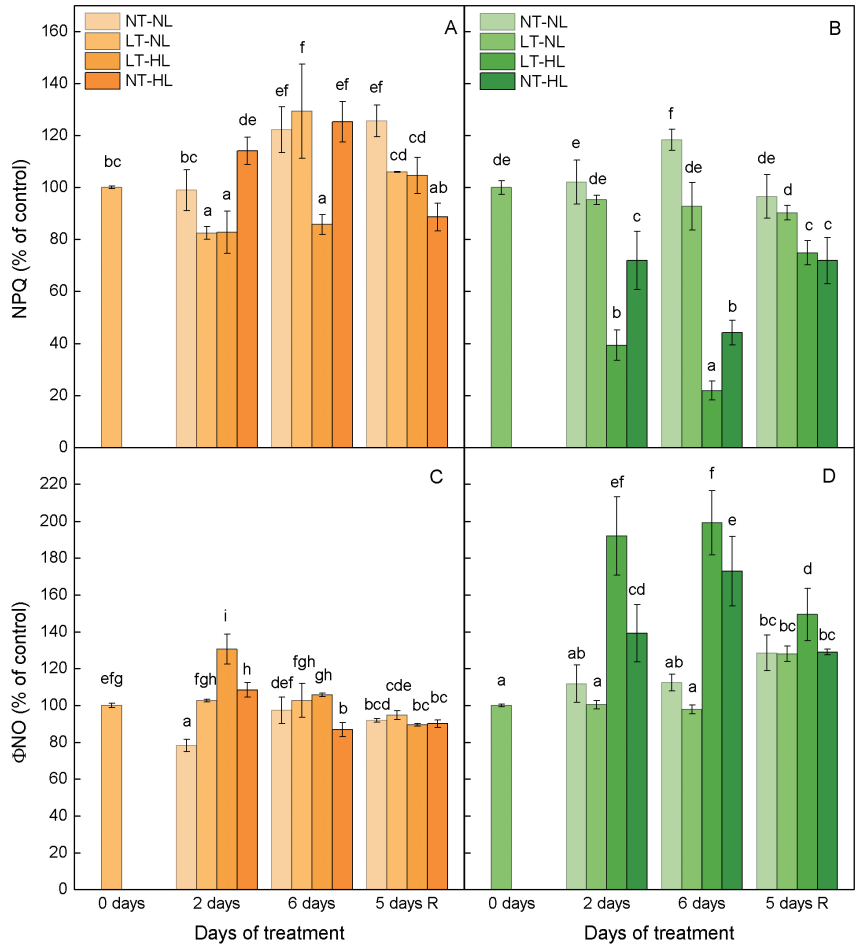


Fig. 3. Alterations of NPQ (A, B) and ΦNO (C, D) of tomato (A, C) and Arabidopsis (B, D) after different treatments. For NPQ 100% corresponds to 1.132 for tomato and 0.652 for Arabidopsis; for ΦNO 100% corresponds to 0.24 for tomato and 0.27 for Arabidopsis

Changes in effectiveness of non-photochemical (NPQ) and non-regulated (ΦNO) quenching. One of the defense mechanisms is the activation of quenching processes of excess energy that was not used for photochemistry. In Fig. 3 data about NPQ and ΦNO were presented as percentage from values for non-treated plants. For tomato the treatment for 6 days by LT–NL resulted in an increase of NPQ and a decrease for LT–HL treated plants. In Arabidopsis HL seemed to have more severe effect on NPQ in comparison with LT. NPQ was inhibited by 60% and 70% after 2 and 6 days, respectively, in LT–HL treated Arabidopsis and only partially recovered, reaching a maximum of 80% of control values. ΦNO showed different behaviour in both plants. There was a small in-

crease of ΦNO in tomato after 2 days at LT–HL, but in Arabidopsis a significant increase by more than 80% was observed after 2 and 6 days treatment by LT–HL.

MDA content and accumulation of anthocyanins. MDA test is routinely used as a measure for membrane injury because of lipid peroxidation. In Fig. 4A, B data about MDA content are presented. For tomato the content of MDA was not changed after 2 and 6 days of treatment by LT–NL but a significant increase is detected at treatment by LT–HL – by about 50% and 100% after 2 and 6 days, respectively (Fig. 4A). On the 2nd day of treatment by LT–HL a difference between both plants was detected – while for tomato plants the increase of MDA was by about 50%, in Arabidopsis the increase was much more evident – by about 230%. The increase of MDA content for Arabidopsis, treated by LT–HL was much stronger expressed on the 6th day and did not reduce during recovery period (Fig. 4B). Data show that more intense peroxidation of membrane lipids occurred in Arabidopsis under LT–HL, indicating more severe membrane damage.

During LT–HL treatment significant changes in the appearance of the plants were observed, related to the change in the colour of leaves on the abaxial and adaxial side indicating accumulation of anthocyanins. The change of colour was more expressed for Arabidopsis than in tomato. In Fig. 4C, D data are presented about the changes in anthocyanin content in leaves of tomato and Arabidopsis. Treatment for 2 days by LT–NL did not lead to significant alterations of anthocyanin content for both plants. However, after 6 days at LT–HL a considerable increase by about 140% was registered for tomato. LT–NL treatment of Arabidopsis resulted in a 5-fold increase of anthocyanins content on the 6th day that remained unchanged during recovery period. Treatment by LT–HL of Arabidopsis resulted in considerable increase of anthocyanins content on the 2nd day – increased by about 500% and much stronger on the 6th day.

Discussion. The responses of tomato and Arabidopsis to low temperature were different in respect to pigment content, accumulation of MDA, synthesis of anthocyanins and effectiveness of PSII. Treatment by LT–NL resulted in a decrease in the amount of chlorophyll by about 20% in tomato, but under these conditions the chlorophyll content in Arabidopsis was not affected. Application of LT–HL led to a strong reduction of Chl $(a+b)$ in Arabidopsis, while higher light appeared to diminish the reduction of chlorophyll in tomato. The reduction of Chl after exposure to low temperature has been reported in tomato plants cv Micro-Tom [11, 17], tomato and rice genotypes with different temperature sensitivity [18]. In Arabidopsis, pigment content was more affected by HL than by LT. Different responses in respect to pigment content and stoichiometry between Arabidopsis and extremophiles species have been reported, suggesting that the modulation of photosynthetic pigment's content was an important process for energy balancing mechanism during adaptation to environment [19]. Although not significantly changed, Fv/Fm showed some variations in tomato and Arabidopsis during LT–HL treatment.

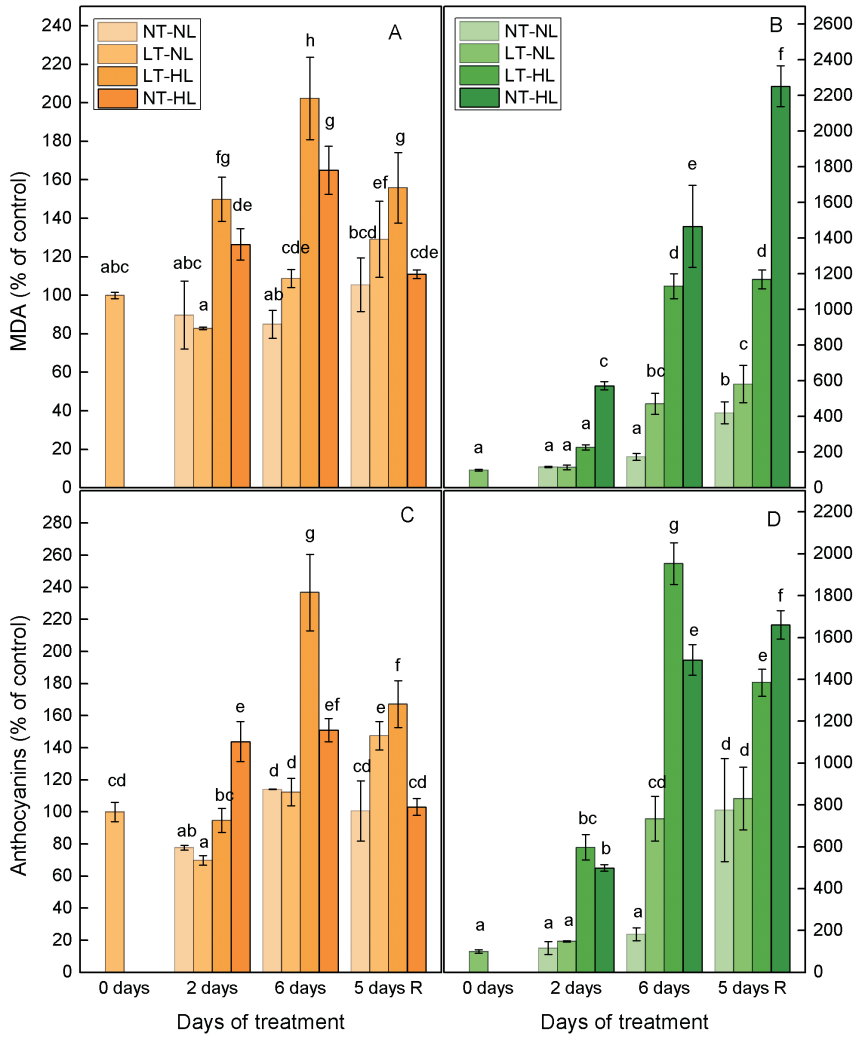


Fig. 4. Effect of treatments on MDA (A, B) and anthocyanins (C, D) accumulation in tomato (A, C) and Arabidopsis (B, D) leaves from control and treated plants. For MDA 100% corresponds to 39.1325 ± 1.805 nmol MDA g^{-1} FW for tomato and to 28.799 nmol MDA g^{-1} FW for Arabidopsis. For anthocyanins 100% corresponds to 0.095 ± 0.003 for tomato and 0.048 ± 0.002 for Arabidopsis

While low temperature considerably affected photosynthesis in tomato, especially on the 6th day, for Arabidopsis no inhibition was detected during treatment by LT-NL. The combination LT–HL expressed an inhibitory effect on O_2 evolution in both plants, more pronounced in tomato. It has been shown that the high light intensified significantly the low temperature-induced inhibition of net photosynthesis in low temperature tolerant *Kobresia pygmaea* Clarke [20]. Our results confirmed that simultaneous action of low temperature and high light had

a stronger negative impact on net photosynthesis and this effect remained after the recovery period. LT–HL increased significantly the excitation pressure on PSII thus resulting in intensification of inhibition [7, 13].

The quenching processes (NPQ and ΦNO) permit the plants to keep the balance between photochemistry and metabolic process and to reduce possible over generation of ROS. We detected very different trends of NPQ and ΦNO in tomato and Arabidopsis during treatment by LT–HL for 6 days. The main difference was that while NPQ was strongly inhibited in Arabidopsis on the 2nd and on the 6th day, no reduction was observed in tomato. However, ΦNO, which is independent on the xanthophyll cycle, increased accordingly, thus fully compensating for the decrease in NPQ. It should be noted here that this difference between tomato and Arabidopsis appeared only for treatment by LT–HL and NT–HL, indicating the role of light intensity for the observed inhibition.

LT treatment had a weak stimulatory effect on MDA accumulation, but LT–HL led to significant increase, more pronounced in Arabidopsis, where the increase is several times higher than in tomato. A similar difference between tomato and Arabidopsis was observed when measuring the accumulation of anthocyanins. The synthesis and accumulation of anthocyanins under LT–HL conditions, significantly more pronounced in Arabidopsis, indicates that high light intensities intensify the stress experienced by plants at low temperatures. MDA and anthocyanin data showed that requirements of both plants to light intensity, i.e. to the excitation energy, were affected to varying degrees by the decrease in temperature. The two plant species' needs for light intensity seem to differ as the temperature drops to suboptimal, which requires further research.

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