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Research article

Tomato plants acclimate better to elevated temperature and high light than to treatment with each factor separately

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ABSTRACT

The influence of two factors – high temperature and high light intensity, acting separately or simultaneously on the pigment composition, fluorescent characteristics, membrane integrity and synthesis of protective substances was investigated in tomato plants (*Solanum lycopersicum* cv. M 82). Moderate elevated temperatures (38/29 °C) were applied under optimum or high light intensity for 2 and 6 days and after that the plants are allowed to recover for 5 days at optimum conditions. Parameters of chlorophyll fluorescence were used to evaluate the alterations of photosystem I and photosystem II activity and malondialdehyde content was determined as a measure of stress-induced peroxidation of membrane lipids. The response of treated plants to high light and elevated temperature was estimated by analyzing the accumulation of anthocyanins. Both stress factors exhibit different impact on studied parameters – high light intensity influences considerably quantum yield of photosystem II and photochemical quenching that is compensated to some extent when applied at elevated temperature. High temperature reduces strongly non-photochemical quenching. Data obtained show that after two days under particular conditions, the plants tend to acclimate, but this is achieved after longer treatment – 6 days. During the recovery period the activity of photosystem I and the quantum yield of photosystem II recover almost completely, while the values of non-photochemical quenching although slightly higher, did not reach the levels at the beginning of treatment.

1. Introduction

The photosynthetic process is very sensitive to extreme temperatures, excess light intensity, UV-radiation, drought etc. The primary photosynthetic reactions which take place in thylakoid membranes of chloroplasts include light absorption, electron transport, oxygen evolution and energy transduction are known to be extremely susceptible to environmental stress conditions (Ashraf and Harris, 2013). Under changed environment in many regions on Earth plants suffer adverse effect of high temperature very often combined with water deficit, high light intensity and drought. To cope with these unfavorable environmental conditions the plants developed various protective strategies through activation of enzymatic defense and accumulation of protective compounds. The influence of each individual factor – high or low temperature, high light intensity, salinity or drought on photosynthesis is intensively studied for variety of plant species and photosynthetic organisms – algae and cyanobacteria in respect to morphological and physiological changes, alterations of primary photosynthetic reactions and of biochemical processes (Wahid et al., 2007 and references herein). The development at elevated temperature causes alterations in structural organization of thylakoids (Karim et al., 1997) and loss of grana stacking or their swelling and formation of antenna depleted PSII (Zhang et al., 2005). Plants differ in respect to their heat tolerance and various threshold temperatures has been reported from different groups as the correct estimation is difficult due to the additional influence of other environmental factors (Wahid et al., 2007). Short time high temperature treatment (2 h at 45 °C) affects the functional activity of photosynthetic apparatus in respect to thermotolerance of two tomato cultivars by different manner (Camejo et al., 2005), the

Abbreviations: chl, chlorophyll; F_o, ground fluorescence in the dark-adapted state; F_v, variable fluorescence; F'_o, ground fluorescence in the light-adapted state; F_m, maximal fluorescence in dark-adapted state; F'_m, maximal fluorescence in light-adapted state; MDA, malonedialdehyde; NPQ, nonphotochemical quenching; PSI (II), photosystem I (II); q_p, photochemical quenching; Φ_{PSII}, quantum yield of photosystem II; ROS, reactive oxygen species; TBA, thiobarbituric acid; TCA, trichloracetic acid.

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inactivation being related to membrane damage and to changes of chlorophyll and carotenoids content. Mild heat treatment of detached leaves significantly stimulated cyclic electron flow around PSI (Havaux, 1996; Bukhov et al., 2000). Exposure of tomato plants for 30 days at 35 °C led to alteration of microstructure of leaves and ultrastructure of chloroplasts thus indicating that one of the important reasons for the observed decrease of net photosynthesis at elevated temperature might be the change in structural organization of photosynthetic apparatus (Zhang et al., 2014). Photosynthetic apparatus is very susceptible to high temperature, photosystem II and oxygen evolving complex being extremely sensitive (Mathur et al., 2014 and references herein). The primary target of damage at high temperature is considered to be the photochemical reactions in thylakoid membranes and carbon metabolism in thylakoid stroma (Wise et al., 2004). As a response to elevated temperature plants express heat shock proteins that could exert protective effect against photoinhibition and to protect PSII from oxidative stress (Stapel et al., 1993; Neta-Sharir et al., 2005).

The impact of high light intensity on plants and other photosynthetic organisms has been widely studied and a number of mechanisms of photoinhibition and photoprotection has been proposed and discussed (Powles, 1984; Aro et al., 1993). All suggested mechanisms of stress-induced damage include the involvement of reactive oxygen species (ROS) that are produced and accumulated under abiotic stress (Asada, 2006). ROS can induce lipid peroxidation and consequently membrane damage, protein degradation and enzyme inactivation (Niyogi, 1999; Sairam et al., 2000). As a response to exposure of plants to high light an accumulation of anthocyanins in vegetative tissues is induced that prevent detrimental effect of excess light on the photosynthetic apparatus which can lead to photoinhibition (Steyn et al., 2002). Anthocyanins are known to accumulate in vacuoles and contribute to light screening, pigmentation and photoprotection via light attenuation and/or antioxidant activity (Hernandez et al., 2009; Landi et al., 2015; Gould et al., 2002; Nagata et al., 2003). *In vitro* anthocyanins show a potent antioxidant activity and their capacity to scavenge ROS are several folds higher than those of ascorbic acid and vitamin E (Gould et al., 2002).

In nature, plants are exposed usually to multiple stress factors – heat stress is accompanied by drought or high light and etc. In the last years researchers are focused on the effect of simultaneous action of two stressors and mechanisms of their impact (Backhausen et al., 2005; Rivero et al., 2014; Buchner et al., 2015; Krause et al., 2015). Recently Buchner et al. (2015) have published results for three high mountain species *Rhododendron ferrugineum*, *Senecioincanus*, and *Ranunculus glacialis* showing that heat treatment applied in the dark reversibly reduced photosynthetic performance and the maximal quantum efficiency of PSII (F_v/F_m). In contrast, plants exposed to heat stress under natural irradiation tolerated and recovered more effectively after treatment. In addition, the critical threshold temperature for chlorophyll fluorescence was higher under illumination than in dark. Krause et al. (2015) have reported that illumination of leaves from two neo-tropical plants *Ficus insipida Willd* and *Calophyllum longifolium* contribute to a small but significant increase in their heat tolerance in comparison with those heated in dark, as determined by the maximal quantum efficiency (F_v/F_m). It has been also shown that long term acclimation of *Arabidopsis thaliana* plants to high light at different temperatures involves regulation of photosystem II antenna size and enhanced energy dissipation (Ballotari et al., 2007). Similar observation has been reported that by regulating the photochemical energy transfer in heat-treated wheat at 40 °C light contributed to heat tolerance of the photosystems (Marutani et al., 2014). These results, concerning the two main environmental stress factors – high temperature and high light, arises the question about the mechanisms of interplay between heat and light tolerance. The significant analogy between the response of photosynthetic apparatus of leaves to mild heating and to strong illumination has been observed and it has been proposed that similar phenomena underlie the short-term adaptation of photosynthesis to heat and to high light (Havaux and Tardy, 1996). It has been demonstrated that heat treatment resulted in a twice increase of the extent of LHCII phosphorylation and in a decrease of the amount of LHCII-related polypeptides in grana membranes in pea plants subjected to high temperature in dark for 15 h (Mohanty et al., 2002).

In the present study tomato plants were subjected to influence of two stress factors – elevated temperature and high light, acting separately or in combination. The design of experiments allowed us to evaluate the effect of temperature and of high light intensity as well as their simultaneous action. The photosynthetic activity was measured in detached leaves from treated up to 6 days plants using pulse amplitude modulated fluorescence (PAM). Stress-induced alterations in the pigment content were determined spectrometrically. MDA test was applied for estimation of lipid peroxidation of treated plants. The relation between ability of plants to cope with unfavorable conditions and accumulation of anthocyanins was examined as well.

2. Materials and methods

2.1. Plant growth conditions

Tomato plants (*Solanum lycopersicum* cv. M 82) were used for all experiments. Seeds were soaked on moist filter paper for 48 h at room temperature, transferred into perlite-containing soil and kept at 4 °C for 4 days. After that pots were transferred to growth chambers and grown under controlled conditions for 22 days: day/night cycle 16 h/8 h at temperature 22/20 °C and illumination with 250 μmol m^{-2} s^{-1}. Fully developed tomato plants at the stage of 5th leave were further grown in growth chambers for 6 days under normal conditions – NT-NL (22/20 °C and 250 μmol m^{-2} s^{-1}), HT-NL (38/29 °C and 250 μmol m^{-2} s^{-1}), NT-HL (22/20 °C and 800 μmol m^{-2} s^{-1}) or HT-HL (38/29 °C and 800 μmol m^{-2} s^{-1}). After 6 days of treatment the plants were returned to normal conditions (NT-NL) and allowed to recover for 5 days. Samples were taken at the end of dark phase of day/night cycle for beginning of experiment (0 day), after 2 and 6 days of treatment at respective conditions and after 5 days of recovery at NT-NL.

2.2. Oxygen evolution of whole leaves

Oxygen evolving activity of control and treated with different combinations of light intensity and temperature plants was polarographically determined using a Clark-type electrode (model *DW1*, *Hansatech Instruments*, King's Lynn, Norfolk, UK) equipped with a LD1/2 leaf-disc electrode chamber. Every measurement is performed on 8 leaf discs with a total area of 10 cm^2 in a saturating atmosphere of CO_2, provided by 200 μl 1 M $NaHCO_3$, at room temperature (22 °C), that corresponds to the growth temperature of plants before temperature/light treatment. The intensity of light is selected from the plateau of light curves of oxygen evolution, because at the intensity from the initial slope of oxygen evolution the values of different samples are not clearly distinguishable. Leaf discs were dark-adapted for 5 min followed by 5 min illumination. For every time point at least 4 measurements were performed.

2.3. Pigment content

Pigment content was determined in 80% acetone (v/v) extract of