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Heat stress and spermidine: effect on chlorophyll fluorescence in tomato plants

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Abstract

Two tomato (*Lycopersicon esculentum* L.) cultivars: Robin (tolerant) and Roma (sensitive to heat stress) were studied. Chlorophyll fluorescence induction parameters (F_V/F_P, A_{max}, and Rfd) at 25 °C showed that the PS2 activity was similar for both cultivars. The parameters, measured at 38 °C, decreased in both cultivars, but more in cv. Roma. Exogenous application of 4 mM spermidine improved the plant heat-resistance in both cultivars, and especially in cv. Roma. Analysis of chlorophyll fluorescence changes during linear increase in temperature showed that cv. Robin plants have higher ability to hardening and higher resistance to thermal damage of the pigment-protein complexes structure and the activity of PS2 than cv. Roma.

Additional key words: chlorophyll *a* fluorescence induction, *Lycopersicon esculentum*, polyamines.

Introduction

High temperature is an important stress factor that modifies the structure and damages the function of biomembranes. Increased temperature induces biosynthesis of heat-shock proteins (HSPs) after a short time, which increases cell thermostability (Vierling 1991, Kislyuk *et al.* 1992, Porankiewicz and Gwóźdź 1993). Thylakoid membranes are particularly sensitive to heat stress, which is expressed in their ultrastructure changes (Thebut and Santarius 1982, Seemann *et al.* 1984) and in inhibition of photosynthesis (Starck *et al.* 1993).

Enhanced biosynthesis of polyamines, the compounds which stabilise the structure of biopolymers and the functions of biomembranes, plays an important role in the protective response of plants to various abiotic stresses (Schuber 1989). During senescence, and under various stress factors, polyamines contribute to the stability of ultrastructure and photosynthetic activity of chloroplasts. The polyamines are also scavengers of free radicals, thus preventing the peroxidation of lipids (Drolet *et al.* 1986, Floryszak-Wieczorek *et al.* 1992a). Heat-shock induced increase in polyamine content can be ascribed as protective response aimed at structural integrity of membrane and cell walls (Edreva *et al.* 1998), and tolerant plants are able to increase total spermidine and spermine pools under heat stress (Roy and Gosh 1996, Bouchereau *et al.* 1999). Increased content of polyamines can preserve the stability of thylakoid membranes and prevent chlorophyll loss under stress (Cohen *et al.* 1979, Besford *et al.* 1993). However, high polyamine concentration inhibits electron transport in PS2 and causes the decrease of the PS1 activity (Cohen *et al.* 1979). It was observed that also exogenously applied spermidine increased resistance of wheat leaf tissue to water stress and of membrane lipids during heat stress (Floryszak-Wieczorek *et al.* 1992a,b).

Chlorophyll fluorescence analysis can serve as a sensitive indicator of thylakoid membranes damage and functional changes of photosynthetic apparatus under high temperature stress (Seemann *et al.* 1984, Bukhov *et al.* 1987, Havaux 1992, Daniel 1997). The aim of this work was to study how exogenously applied spermidine

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Abbreviations: A_{max} - area above the fluorescence induction curve; F_0 - minimal fluorescence level; F_p - maximal attainable fluorescence level; F_v - variable fluorescence; F_s - stationary fluorescence level; LED - light emitting diode; PAR - photosynthetically active radiation; PS - photosystem.

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influenced chlorophyll fluorescence induction and how heat hardening affected the course of fluorescence temperature curve (FTC) in the leaves of tomato under heat stress.

Materials and methods

The objects of the study were the second or third leaves of two tomato (*Lycopersicon esculentum* L.) cultivars: Robin (tolerant) and Roma (sensitive to heat stress). The plants were grown for 25 d in laboratory conditions in plastic pots filled with sand and watered with 1:1 water diluted Hoagland nutrient solution, illuminated with LRFR 400W mercury lamps [irradiance (PAR) at the leaf level of 160 μmol(photon)$m^{-2}s^{-1}$, photoperiod 14 h, day/night temperature 24/18 °C, and relative humidity 70 %)]. The plants of both cultivars, being at the stage 3 to 4 leaves, were divided into two groups, of which one (NH - unhardened) remained in the above described conditions. The plants of other group (H - hardened) were placed in the thermoluminostate chamber, being gradually heated from 25 to 38 °C, at a controlled rate of 1 °C per 8 min. The conditions in the thermoluminostate chamber, where the plants remained for 3 d, were the following: irradiance 160 μmol m^{-2} s^{-1}, photoperiod 14 h, day/night temperature 38/30 °C, and relative humidity 80 %.

The measurements of chlorophyll fluorescence induction, as well as dependence of fluorescence on temperature, were carried out with a home-made computer steered fluorometer (Fig. 1). Red irradiance (LED; λ_{max} = 660 nm) of 125 μmol m^{-2} s^{-1}, was used for excitation and fluorescence was measured at $\lambda > 690$ nm.

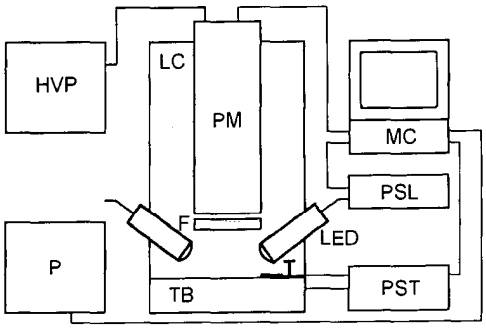

Fig. 1. Block diagram of the system for fluorescence measurements: F - filter ($\lambda > 690$ nm), HVP - high voltage power supply, LC - light-proof camera, LED - light emitting diode (λ_{max} = 660 nm), MC - microcomputer system, P - printer, PM - photomultiplier, PSL - power supply for LED, PST - power supply for thermoelectric battery, T - electric thermometer, TB - thermoelectric battery.

Duration of fluorescence measurements was 240 s. The following parameters were calculated: F_v/F_p - the coefficient of photochemical reaction efficiency in PS2; A_{max} - area above the fluorescence induction curve, the parameter informing about the integral quantity of electron acceptors between PS2 and PS1 (Lavorel *et al.* 1986); $Rfd = (F_p - F_s)/F_s$ informs about the interaction and equilibrium between primary photosynthetic reactions and dark enzymatic reactions, referred to as "vitality index" (Lichtenthaler *et al.* 1986); variable fluorescence $F_v = F_p - F_0$; F_p - fluorescence at P-level (highest attainable fluorescence); F_0 - minimal fluorescence resulting from the loss of excitation energy during its migration within pigment antenna (Lichtenthaler and Rinderle 1988); F_s - fluorescence in steady state.

Fluorescence temperature curve (FTC; Fig. 2) represents changes in fluorescence during plant linear heating (Pospíšil *et al.* 1998). Chlorophyll fluorescence was induced with weak red irradiance of 6 μmol m^{-2} s^{-1} (LED; λ_{max} = 660 nm), temperature of the samples was elevated within the range 30 - 60 °C at the rate 1 °C per 20 s, and the entire measurement cycle was controlled with use of the *ATEM* computer programme.

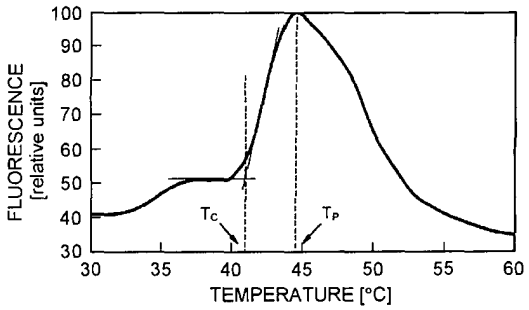

Fig. 2. Fluorescence temperature curve; T_c - critical temperature for heat-induced fluorescence rise, T_P - temperature of heat-induced peak fluorescence.

To calculate the least significant differences, the Student *t*-test was used.

In the first experiment, a part of the unhardened plants of cvs. Robin and Roma (12 plants from each cultivar) were taken from the pots with their roots rinsed from sand and placed in pots with half strength Hoagland nutrient solution (C plants) or 4 mM of spermidine in the same solution for 48 h, and after that the measurements of chlorophyll fluorescence induction were carried out. Discs of young, developed leaves (11 mm diameter) were cut, placed on a thermostabilised plate (temperature 25 °C), covered with a glass plate, and put in the fluorometer chamber. After 20 min of dark-adaptation, the chlorophyll *a* fluorescence induction was recorded and three parameters (F_v/F_P, A_{max} and Rfd) were calculated. Then the discs remained in the chamber in darkness, being gradually heated up at the controlled rate

of 0.5 °C per 30 s, from 25 to 38 °C. After 20 min of dark-adaptation at this temperature, the chlorophyll *a* fluorescence induction records were repeated and the above mentioned three parameters were estimated again.

In the second experiment, discs of 11 mm in diameter were cut from young, developed leaves of the plants which were heat-hardened (H) and placed on the thermostabilised plate (temperature 30 °C), covered with a glass plate, and put in the fluorometer chamber in order to measure the fluorescence temperature curve (FTC) in the range from 30 to 60 °C, at a rate of 0.5 °C per 10 s. The measurements were carried out in six repetitions. The same measurements set was conducted with unhardened plants (NH).

Results and discussion

The results confirm the possibility of the photosynthesis inhibition by spermidine (Cohen *et al.* 1979). The parameters of chlorophyll fluorescence induction were nearly equal in control plants of both tomato cultivars. The exogenously applied 4 mM spermidine slightly decreased chlorophyll fluorescence induction parameters. While the decrease in F_v/F_P and Rfd were statistically insignificant, the reduction of plastoquinone pool (PQ), which was indicated by the decrease of A_{max} was 23 and 12 % for Robin and Roma, respectively (Table 1).

Table 1. Chlorophyll fluorescence induction parameters (estimated at 25 °C) as affected by application of 4 mM spermidine for 48 h; mean ± SD, * - differences significant at $P = 0.05$.

Cultivar	F_v/F_p		A_{max}		Rfd	
	control	spermidine	control	spermidine	control	spermidine
Robin	0.753 ± 0.017	0.723 ± 0.030	55.5 ± 4.9	42.7 ± 4.3*	2.64 ± 0.11	2.51 ± 0.17
Roma	0.750 ± 0.025	0.734 ± 0.018	54.6 ± 3.2	48.1 ± 2.3*	2.46 ± 0.17	2.35 ± 0.23

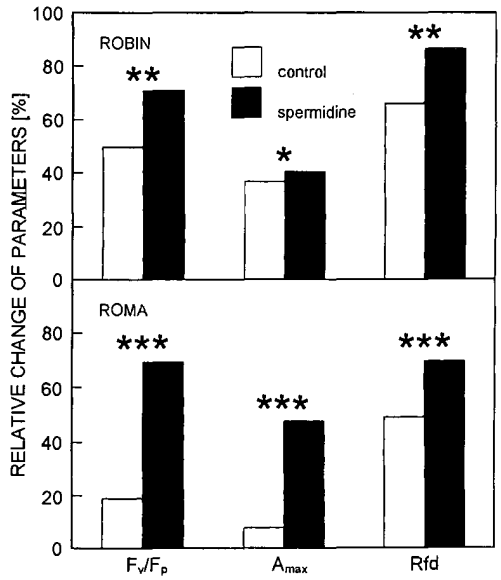

Fig. 3. Relative change of chlorophyll fluorescence induction parameters in leaves of non-hardened tomato plants cvs. Robin and Roma at 38 °C. The values of parameters estimated at 25 °C were considered as 100 %. Detailed description of measurements in text. *, **, *** - differences significant at $P = 0.05$, 0.01, and 0.001, respectively.

The strong inhibition of light phase photosynthesis occurred at 38 °C, which demonstrated the decrease in PS2 activity and the reduction of PQ for both cultivars. The decrease of the parameters F_v/F_P and A_{max} was more distinct for thermosensitive cv. Roma than for the tolerant cv. Robin. Also the Rfd in the Roma exhibited over 50 % decrease in relation to its value determined at 25 °C, which indicates the disturbance the potential photosynthetic activity of leaves (Lichtenthaler and Rinderle 1988). The impact of heat stress on the photosynthetic reactions in cv. Roma plant leaves can be explained by previously described changes in chloroplast ultrastructure (Starck *et al.* 1993). The exogenously applied spermidine to high degree alleviated the high temperature impact in both tomato plants cultivars, which can be explained with a general ability of polyamines to stabilize thylakoid membranes and Calvin cycle enzymes in stress conditions (Schuber 1989). The protective effect of spermidine to photosynthesis reactions at 38 °C was especially evident in cv. Roma, which may probably suggest that their thermosensitivity could result from a low concentration of endogenous substances protecting biomembranes under heat stress conditions (Volger and Santarius 1981). This suggestion, however, should be confirmed by additional studies.

FTC analysis is a widely used method for examination of damage of PS2 centres and associated pigment-protein complexes (Seemann *et al.* 1984, Havaux 1992, Pospíšil *et al.* 1998). Critical temperature (T_c) in FTC plot (Fig. 4) correlates with the beginning of thermal denaturation of PS2 (Seemann *et al.* 1984) and temperature T_P (peak FTC) corresponds to complete destruction of PS2 and irreversible inhibition of

photosynthesis (Terzaghi *et al.* 1989). The values of T_C and T_P are linked to the general tolerance of the plants to thermal stress (Bilger *et al.* 1984). The course of FTC during rapid heating of unhardened leaf tissue (3 °C per min) did not show significant differences in thermal sensitivity between the both cultivars studied. After heat hardening, an increase in resistance to thermal denaturation of PS2 and the temperature complete inhibition of photosynthesis appeared (T_P increased). The increase in the heat-resistance was considerably higher for the cv. Robin than for cv. Roma. Lower ability of cv. Roma to heat hardening is probably the main reason of its weak resistance to heat stress.

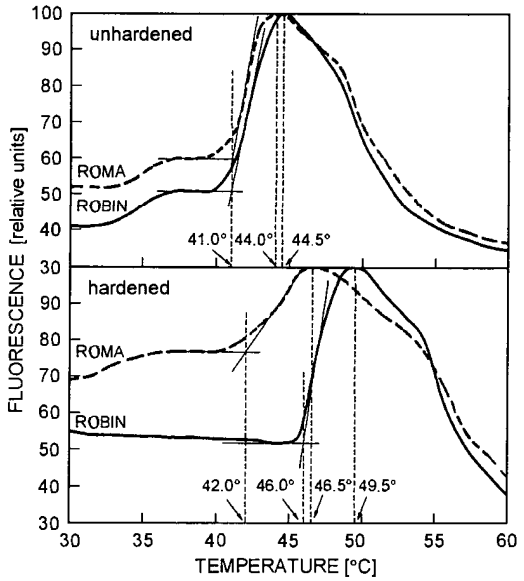

Fig. 4. Heat-induced changes in fluorescence temperature curve in tomato leaves of cvs. Robin and Roma. Leaf discs were heated up at the rate 0.5 °C per 10 s. Details are in text.

Presented studies may suggest that the plants of the studied cultivars have similar photosynthetic activity at 25 °C as demonstrated by the analysis of chlorophyll fluorescence induction curves. The increase of leaf tissue temperature to 38 °C causes a considerable decrease in the PS2 activity in cv. Roma and moderate decrease in PS2 activity in cv. Robin. The exogenously applied spermidine had protecting effect on the decrease of of PS2 activity and Rfd value, especially in cv. Roma.

The changes of chlorophyll fluorescence induction of leaves and FTC can serve as practical indicators of damages to thylakoid membranes, PS2 reaction centres activity, and the pigment-protein complexes structure.

Reference

Besford, R.T., Richardson, C.M., Capell, T., Tiburcio, A.F.: Effect of polyamine on stabilization of molecular complexes in thylakoid membranes on osmotically stressed oat leaves. - Planta **189**: 201-206, 1993.

Bilger, H.W., Schreiber, U., Lange, O.L.: Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. - Oecologia **63**: 256-262, 1984.

Bouchereau, A., Azis, A., Larher, F., Martin-Tanguy J.: Polyamines and environmental challanges: recent development. - Plant Sci. **140**: 103-125, 1999.

Bukhov, N.G., Dzhibladze, T.G., Karapetyan, N.V.: [Aftereffects of high temperatures on the kinetics of variable and delayed fluorescence in leaves.] - Fiziol. Rast. **34**: 435-444, 1987. [In Russ.]

Cohen, A.S., Popovic, R.B., Zalik, S.: Effects of polyamines on chlorophyll and protein content, photochemical activity and chloroplast ultrastructure of barley leaf discs during senescence. - Plant Physiol. **64**: 717-720, 1979.

Daniel, E.: The temperature dependence of photoinhibition in leaves of *Phaseolus vulgaris* (L.). - Plant Sci. **124**: 1-8, 1997.

Drolet, G., Dumbroff, E.B., Legge, R.L., Thompson, J.E.: Radical scavenging properties of polyamines. - Phytochemistry **25**: 367-371, 1986.

Edreva, A., Yordanov, I., Kardijeva, R., Gesheva, E.: Heat shock responses of bean plants: involvement of free radicals, antioxidants and free radical/active oxygen scavenging systems. - Biol. Plant. **41**: 185-191, 1998.

Floryszak-Wieczorek, J., Grabikowski, E., Kubiś, J., Krzywański, Z.: The effect of spermidine on lipid peroxidation in wheat leaves during water stress. - Acta Physiol. Plant. **14**: 3-10, 1992a.

Floryszak-Wieczorek, J., Murkowski, A., Kubiś, J., Krzywański, Z.: The effect of spermidine on chlorophyll luminescence in wheat leaves under water stress. - Acta Physiol. Plant. **14**: 119-126, 1992b.

Havaux, M.: Stress tolerance of photosystem II *in vivo*. - Plant Physiol. **100**: 424-432, 1992.

Kislyuk, L.M., Bubolo, L.S., Paleeva, T.V.: [Heat shock effects on primary thermostability and reparability of photosynthesis and on the ultrastructure of mesophyll cells in strawberry *Fragaria vesca* L.] - Fiziol. Rast. **39**: 939-948, 1992. [In Russ.]

Lavorel, J., Breton, J., Lutz, M.: Methodological principles of measurements of light emitted by photosynthetic systems. - In: Govindjee, Amesz, J., Fork, D.C. (ed.): Light Emission by Plants and Bacteria. Pp. 57-98. Academic Press, Orlando 1986.

Lichtenthaler, H., Buschmann, C., Rinderle, U., Schmuck, G.: Application of chlorophyll fluorescence in ecophysiology. - Radiat. Environ. Biophys. **25**: 297-308, 1986.

Lichtenthaler, H., Rinderle, U.: The role of chlorophyll fluorescence in the detection of stress conditions in plants. - Crit. Rev. anal. Chem. **19**: 29-85, 1988.

Porankiewicz, J., Gwóźdź, E.A.: [Heat shock proteins and their role in plant cell.] - Post. Biol. Komórki **20** (2): 155-170, 1993. [In Polish.]

Pospíšil, P., Skotnica, J., Nauš, J.: Low and high temperature dependence of minimum F_0 and maximum F_m chlorophyll

fluorescence *in vivo*. - Biochim. biophys. Acta **1363**: 95-99, 1998.

Roy, M., Gosh, B. Polyamines, both common and uncommon, under heat stress in rice (*Oryza sativa*) callus. - Physiol. Plant. **98**: 196-200, 1996.

Schuber, F.: Influence of polyamines on membrane function. - Biochem. J. **260**: 1-10, 1989.

Seemann, J.R., Berry, J.A., Downton, W.J.S.: Photosynthetic response and adaptation to high temperature in desert plants. A comparison of gas exchange and fluorescence methods for studies of thermal tolerance. - Plant Physiol. **75**: 364-368, 1984.

Starck, Z., Ważynska, Z., Kucewicz, O.: Comparative effects of heat stress on photosynthesis and chloroplast ultrastructure in tomato plants with source-sink modulated by growth regulators. - Acta Physiol. Plant. **16**: 125-133, 1993.

Terzaghi, W.B., Fork, D.C., Berry, J.A., Field, C.B.: Low and high temperature limits to PSII. - Plant Physiol. **91**: 1494-1500, 1989.

Thebut, R., Santarius, K.A.: Effects of high temperature stress on various biomembranes of leaf cell *in situ* and *in vitro*. - Plant Physiol. **70**: 200-205, 1982.

Vierling, E.: The roles of heat shock protein in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 579-620, 1991.

Volger, H., Santarius, K.A.: Release of membrane proteins in relation to heat injury of spinach chloroplast. - Physiol. Plant. **51**: 195-200, 1981.