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 (Fv/Fp, Amax, and Rd) 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 thermotolerance (Vierling 1991, Kislyuk et al. 1992, Porankiewicz and Gwoźdź 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
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(photons) 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 thermostate chamber, being gradually heated from 25 to 38 °C, at a controlled rate of 1 °C per 8 min. The conditions in the thermostate 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-stereotyped fluorometer (Fig. 1). Red irradiance (LED; λ<sub>max</sub> = 660 nm) of 125 μmol m⁻² s⁻¹, was used for excitation and fluorescence was measured at λ > 690 nm.

![Block diagram of the system for fluorescence measurements](image)

**Fig. 1.** Block diagram of the system for fluorescence measurements: F - filter (λ > 690 nm), HVP - high voltage power supply, LC - light-proof camera, LED - light-emitting diode (λ<sub>max</sub> = 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: Fₐ/Fₚ - the coefficient of photochemical reaction efficiency in PS2; Αₚₜₐₜ - 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 = (Fₚ₋Fₖ)/Fₚ 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<sub>v</sub> = Fₚ - Fₖ; F<sub>p</sub> - fluorescence at P-level (highest attainable fluorescence); F<sub>d</sub> - minimal fluorescence resulting from the loss of excitation energy during its migration within pigment antenna (Lichtenthaler and Rinderle 1988); F inherent 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<sub>max</sub> = 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.

![Fluorescence temperature curve](image)

**Fig. 2.** Fluorescence temperature curve; T<sub>e</sub> - critical temperature for heat-induced fluorescence rise, T<sub>p</sub> - 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. Disks of young, developed leaves (11 mm diameter) were cut, placed on a thermostabilized 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<sub>v</sub>/F<sub>p</sub>, Αₚₜₐₜ, and Rfd) were calculated. Then the discs remained in the chamber in darkness, being gradually heated up at the controlled rate.