Chlorophyll *a* fluorescence OJIP transient as a tool to characterize and evaluate response to heat and chilling stress in tomato leaf and fruit

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ABSTRACT

In tomato production, extreme temperatures such as heat and chilling are major factors limiting growth and productivity. Recently, the chlorophyll *a* fluorescence OJIP transient (OJIP transient) has been used as an effective tool for studying damage to and activity of the electron transport chain in the photosynthetic apparatus under various environmental stresses. The aim of this study was to evaluate the validity of the OJIP transient as a stress indicator and to characterize the effect of heat and chilling stress on the photosynthetic apparatus in tomato leaf and fruit. Detached leaves and fruits were incubated at 25 °C (control), 40 °C (heat stress), and 4 °C (chilling stress); OJIP transients were measured after exposure to stress for 1 h and 24 h, and several parameters were calculated according to the JIP-test. After exposure to stress for 1 h and 24 h, the OJIP curves and the JIP parameters clearly revealed differences between stress types and between tissue types. In addition, the JIP parameters and the energy pipeline model indicated that heat stress had a greater influence on the photosystem (PS) II electron transport chain than chilling stress, and that changes were greater in the fruit than in the leaf. Furthermore, the PS I electron transport chains of leaf and fruit appeared to be more heat resistant than those in PS II. Our results indicate that, in tomato leaf and fruit, OJIP transients and calculated JIP parameters can be used as sensitive methods for measuring the heat and chilling stress damage to the photosynthetic apparatus, and to identify the action sites of temperature stress.

1. Introduction

Tomato is one of the most important horticultural crops in the world. The cultivated tomato originated and was domesticated in tropical and subtropical regions, but commercial tomato production is extensive in many temperature zones (Foolad, 2005). In general, temperatures higher or lower than optimal plant growth requirements can markedly disrupt many essential metabolic processes. The tomato requires optimum temperatures of 22–26 °C for growth and development, and exposure to higher temperatures can markedly alter several metabolic processes. For example, photosynthetic rate in tomato leaf was reduced by a brief (20 min) increase in leaf temperature to 35 °C (Camejo et al., 2010) and a dark treatment plus heat shock (Camejo et al., 2006). In addition, when tomato plants were exposed to a heat-shock temperature of 45 °C for 3 h, heat injury in tomato leaf may involve chlorophyll photooxidation mediated by activated oxygen species and

more severe alterations in the photosynthetic apparatus (Camejo et al., 2007). When exposed to temperatures below 10 °C, most cultivated tomato plants suffer chilling injury, and extended exposure below 6 °C can destroy the plants (Park et al., 2004). Therefore, extreme temperatures during growth are major factors limiting growth and yield in tomato production. To avoid these effects of heat and chilling stress, it is important to evaluate the degree of damage and change in physiological behavior induced by stress in tomato plants.

For evaluating the degree of temperature-related stress damage, the most sensitive component is the photosynthetic apparatus (Rachmilevitch et al., 2006). Recently, changes in the chlorophyll *a* fluorescence OJIP transient (OJIP transient) have been used to evaluate the extent of damage to the photosynthetic apparatus under several environmental stresses. The OJIP transient is defined by O, J, I, and P steps, corresponding to the redox states of photosystem (PS) II and PS I and to the efficiencies of electron transfer through the intersystem chain to the end electron acceptors at the PSI acceptor side (Strasser et al., 2000, 2004). In addition, the OJIP transient has useful and practical advantages: it is non-destructive, easy, and allows rapid testing of any type of chlorophyll-containing sample in any form (Strasser et al., 2000, 2004). Several researchers have reported that the measurement of OJIP transients is a

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sensitive and reliable method for the detection and quantification of heat or chilling-induced changes in the PS II and PS I of plants (Chen and Cheng, 2009; Stefanov et al., 2011; Strasser et al., 2000, 2004; Strauss et al., 2006). For example, changes in OJIP transients caused by high temperature have been used to study heat-tolerance among several common bean cultivars (Stefanov et al., 2011) and apple leaves and peels (Chen et al., 2008, 2009; Chen and Cheng, 2009). Chilling tolerance in soybean genotypes can be evaluated by the OJIP transient of dark chilling stress in soybean (Strauss et al., 2006). However, in tomato plants, it is unclear whether the OJIP transient can be used as a stress indicator and how photosynthetic physiological responses change under heat and chilling stress.

In contrast, most studies analyzing the effects of heat or chilling stress on OJIP transients have been conducted in plant leaves (Ogweno et al., 2009; Petkova et al., 2007; Stefanov et al., 2011; Strauss et al., 2006; Wahid et al., 2007), but in fruits limited only to apple (Chen et al., 2008, 2009; Chen and Cheng, 2009). For example, the comparison of thermotolerance of sun-exposed peel and shaded peel of apple fruit (Chen et al., 2008, 2009) and difference between leaves and peels with regard to the effect of heat stress on PSII (Chen and Cheng, 2009) were evaluated using OJIP transients. In tomato fruit, the pericarp is photosynthetically active, because the pericarp is green and chlorophyll is present from the immature stage until pigmentation. Furthermore, photosynthetic activities are different between leaves and fruits; in the pericarp of cherry tomato, photosynthetic fixation of $^{14}CO_2$ has been shown to occur at higher rates than in the leaves (Laval-Martin et al., 1977). Thus, under heat or chilling stress, changes in the photosynthetic apparatus may differ between leaf and fruit. In apple leaf and peel under heat stress (44 °C), using OJIP transients, the tolerance of PS II to high temperature was greater in the leaf than in the peel (Chen and Cheng, 2009). However, to our knowledge, the effects of heat and chilling stress on the photosynthetic apparatus in tomato leaf and fruit and their differences have not been elucidated.

The aim of this study was to investigate whether the OJIP transient can be used as a reliable indicator of temperature stress in tomato leaf and fruit. In addition, on the basis of various parameters from OJIP transients, we attempted to explain tissue- and stress-specific differences in heat- and chilling-stress responses of the photosynthetic apparatus of tomato leaf and fruit.

2. Materials and methods

2.1. Plant materials

Cherry type tomato plants (*Solanum lycopersicon* cv. Mini Carol) were grown under a glasshouse at the Prefectural University of Kumamoto from April to October 2009. Seeds were sown on vermiculite in an incubator at 28 °C and seedlings with two true leaves were transplanted to 12-cm-diameter pots filled with pumice in the glasshouse. When inflorescence appeared, plants were transplanted into a closed irrigation system consisting of a culture bed filled with pumice (particle size, approximately 5 mm), a 100 L tank for nutrient solutions, and a timer for irrigation. The plants were fertilized with a nutrient solution (half-strength Otsuka-B solution; Otsuka Chemical Co., Osaka, Japan). The pH of the nutrient solutions was measured daily, and when necessary, corrected with 1.0 M H_2SO_4 to maintain the pH at 6.5–7.0. Nutrient solutions were renewed at 7-day intervals. All the plants were topped just below the fourth truss and all lateral shoots were removed periodically.

2.2. Temperature stress

In an experiment using whole plant, the effect of temperature stress on OJIP transients of tomato leaf and fruit may be subject to the influences from other organs such as root. Thus, to prevent the influence of other organs, our study used the detached leaf and fruit. However, the use of detached organs may have an additional effect such as changes in water content. Therefore, to prevent the additional effect, we performed the adaptation of water content in the detached leaf and fruit before temperature stress as described below.

For heat and chilling stress, mature leaves between the first and second trusses and green fruit (about 30 days after flowering) on the first and second trusses were obtained from plants in the morning. To negate the effects of light and temperature differences during growing and the adaptation of the water status in the detached leaf and fruit, detached leaves and fruits wrapped with wet paper towels were incubated at 25 °C for 24 h in the dark in an incubator (MTI-202, EYELA, Tokyo, Japan) before temperature stress treatments. Thereafter, chilling and heat stresses were carried out by transferring to flat plastic trays (33 cm × 25 cm × 6 cm depth) placed in the incubator or a freezer (MPR-311D, SANYO, Tokyo, Japan) set to 40 °C for heat stress, 4 °C for chilling stress, and 25 °C for control, under dark conditions. During treatments, leaf and fruit were wrapped with wet paper towels to prevent drying out in the trays. Each experiment was carried out in two replications ($n \geq 20$).

2.3. Measurement of OJIP transients

OJIP transients were measured after exposure to stress for 1 h and 24 h. Before measurements, leaf and fruit were equipped with special plastic clips (provided by Opti-science, Inc., NH, USA). OJIP transients were measured using a portable chlorophyll *a* fluorometer (OS-30P; Opti-science, Inc., NH, USA). Measurements were carried out on the adaxial leaf lamina and the fruit equator. The OJIP transient was induced by red light of approximately 3000 $\mu mol\ m^{-2}\ s^{-1}$ provided by an array of 3 light-emitting diodes (peak 660 nm). The fluorescence signals were recorded in a time scan from 20 μs to 1 s after the onset of irradiation with a data acquisition rate of 100 readings $m\ s^{-1}$ for 2 ms and 1 reading $m\ s^{-1}$ after 2 ms.

The measured data was used for the calculation according to the JIP-test equations (Chen and Cheng, 2009; Strasser et al., 2000, 2010, 2004). The following fluorescence intensity values from the original measurements were used: minimal intensity at 20 μs, when all PS II reaction centers (RC) are open (the O step); intensity at 300 μs used for calculation of the initial slope (Mo), defined as the net ratio of reaction center (RC) closure; the intensity at 2 ms (the J step); the intensity at 30 ms (the I step); and the maximal intensity when all PS II RCs are closed (the P step = F_M).

The biophysical parameters derived from the OJIP transients were calculated, and the following parameters, which refer to time zero (onset of fluorescence induction) were used. (1) Flux ratio of PS II: φ_{P_0}, the maximum quantum yield of primary photochemistry; Ψ_0, the probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-; φ_{E_0}, the quantum yield of electron transport; and φ_{D_0}, the probability that an absorbed photon is dissipated. (2) Flux ratios of PS I: δ_{R_0}, the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PS I end electron acceptors; φ_{R_0}, the quantum yield of electron transport from Q_A^- to the PS I end electron acceptors; and ρ_{R_0}, the efficiency with which a trapped exciton can move an electron into the electron transport chain from Q_A^- to the PS I end electron acceptors. (3) Specific energy fluxes per RC; absorption (ABS/RC); electron transport (ET_0/RC); trapping (TR_0/RC); dissipation (DI_0/RC); and reduction of end acceptors at the PS I electron acceptor side (RE_0/RC). (4) Phenomenological energy fluxes per excited cross section (CS_M, subscript M refer to time F_M): absorption (ABS/$CS_M = F_M$); electron transport (ET_0/CS_M); trapping (TR_0/CS_M;