CHANGES INDUCED BY HIGH TEMPERATURES IN PHOTOSYNTHESIS AND ANTIOXIDANT RESPONSE ON TWO GENOTYPES OF TOMATO (*Lycopersicon esculentum*)

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ABSTRACT. Two tomato genotypes with different sensitivity to high temperatures (cv Amalia and the wild type Nagcarlang) were exposed to several stress conditions (45^{0}C-2 hour exposition, 45^{0}C-3 hour exposition and 25^{0}C as control). Gas exchange and chlorophyll fluorescence were determined as well as antioxidant response expressed by SOD and APX activities. Physiological and biochemical variables changed depending on the genotype and stress exposure time. Gas exchange was reduced by stress conditions (45^{o}C-3 hours), while chlorophyll fluorescence was reduced since the first hours of imposed stress in cv Amalia. The variables evaluated might be useful to describe genotype tolerance to high temperatures.

Key words: photosynthesis, chlorophylls, antioxidants, tomato, heat stress

RESUMEN. Dos cultivares de tomate con distinta susceptibilidad a las altas temperaturas (cv Amalia y el tipo silvestre Nagcarlang) fueron expuestos a diferentes condiciones de estrés (45^{o}C-2horas, 45^{o}C-3horas y 25^{o}C como control). El intercambio gaseoso y la fluorescencia de clorofila fueron determinados así como la respuesta antioxidante expresada por la actividad SOD y APX. Las variables fisiológicas y bioquímicas evaluadas fueron modificadas dependiendo del genotipo y el tiempo de exposición al estrés. El intercambio gaseoso fue reducido por la condición de estrés (45^{o}C-3horas), mientras la fluorescencia de clorofila fue reducida desde las primeras horas de impuesto el estrés en el cv Amalia. Las variables evaluadas pueden ser útiles para describir genotipos tolerantes a las altas temperaturas.

Palabras clave: fotosíntesis, clorofilas, antioxidantes, tomate, estrés térmico

INTRODUCTION

The photosynthetic capacity of leaves is strongly affected by temperature. However, the dependence of each photosynthesis-limiting process on temperature is not necessarily the same. With short-term (minutes to hours) temperature changes, some key components of the photosynthetic apparatus seem to be more affected than others (1). For example, PSII is often thought to be the most labile and easily damaged component of photosynthesis during heat stress (1, 2, 3). Export of photoassimilates is another metabolic process that is sensitive to inhibition by high temperature (4).

There is evidence that inactivation of Rubisco is an early event in the inhibition of photosynthesis by elevated temperature (5) and that inhibition of Rubisco activase may be a key regulatory process affected by high temperature stress (6, 7, 8). However, other reports have concluded that the primary site of high temperature damage is associated with a component of the thylakoid membranes (1, 2, 9).

The operation of a complicated and highly specific mechanism, including the enzymatic and low molecular weight antioxidant systems, serves to maintain the steady-state physiological level of free-radical processes in the cell (10). In plants, the most intense formation of reactive oxygen occurs in chloroplasts, the majority of superoxide radicals are generated during the operation of the electron-transport chain of photosynthesis; the reactive oxygen radicals are generated in PSI (10).

The objective of this work was to study the effect of high temperature *in vivo* on photosynthetic apparatus and the mechanism involved in a possible tolerance, in two tomato genotypes. For this purpose, gas exchange and chlorophyll fluorescence was studied under stress conditions. SOD and APX activities in a chloroplast rich extract were also examined. Special emphasis was made on the role of the antioxidant activities in protection of the photosynthetic apparatus under high temperature conditions.

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MATERIALS AND METHODS

Plant material. Seedlings of two tomato genotypes (*Lycopersicon esculentum*): Amalia, which was obtained by breeding program at the National Institute of Agricultural Sciences, with a good behavior mainly under nonoptimal conditions (11), and the wild thermotolerant type Nagcarlang (12) were grown in pots filled with silica sand and daily irrigated with nutritive solution.

The seedlings were transferred to a growth chamber two weeks before sampling. The plants were grown under a photoperiod of 16/8h light/dark and 25/20ºC. Light intensity was 250 μmol photons.m^{-2}.s^{-1} and relative humidity was 60 %.

At the stage of fourth true leaves, a group of plants were exposed to temperature stress 45ºC for two and other for three hours (45ºC-2h, 45ºC-3h). A group was maintained untreated at 25ºC, which served as control. The experiment was carried out under good irrigation conditions.

The physiological and biochemical measurements were made immediately after stress. Plants were collected and sampled at 9 am and gas exchange and fluorescence measurements were made at 25ºC.

Photosynthesis and chlorophyll fluorescence measurements. At the end of temperature stress, gas exchange measurements were made on the third leaf, from apical to base, using a portable LICOR Li-6200 at 25ºC and light intensity of 250 μmol.m^{-2}.s^{-1}.

Chlorophyll fluorescence was measured on the same leaves used for gas exchange, using a portable Opti-Sciences mod. OS 30 fluorometer. Stressed and control leaves were predarkened for 20 min before starting the experiment. The saturation pulse to determine the emission of fluorescence on the upper leaf surface was 2600 μmol m^{-2}.s^{-1}.

The values of initial fluorescence (Fo) and maximal fluorescence (Fm) were read in the fluorometer. The photochemical efficiency of photosystem II (PSII) (Fv/Fm) and nomenclature used followed Van Kooten and Snel (13).

Chloroplast rich extract. The overall procedure was carried out at 0 to 4ºC. Leaf tissue was carefully mashed in a mortar with extraction medium containing Mops-KOH (30nM pH 7.5), mannitol (350mM), cysteine (1mM) and BSA (0.2%). Homogenate was squeezed through two layers of muslin and centrifuged at 2200g for 30 sec. in a Beckman mod. J2-21 centrifuge. The chloroplast rich precipitate was carefully removed with a brush and resuspended in a medium containing Mops-KOH (30mM pH 7.5), mannitol (350mM) and cysteine (1mM) and centrifuged at 2200g for 30 sec. The precipitate obtained was carefully removed and resuspended in a minimum volume of medium.

Proteins were determined from chloroplast rich extract according to Bradford's method (14) using BSA as a standard.

Superoxide dismutase activity. This activity (SOD) was assayed as previously described by McCord and Fridovich (15). The method is based on the inhibition, by SOD, of citocrom c reduction by free radical $^{\cdot}O_2$, generated by enzymatic xantine-xantine oxidase system. SOD activity was determined in 3 mL reaction mixture containing potassium phosphate (pH 7.8), $EDTANa_2$, citocrom c 1mM, KCN (10mM), and 25-100μL sample.

Reaction was initiated by the addition of xantine-oxidase and the reduction of citocrom c was monitored at 550nm. The enzymatic activity was expressed in unit x mL^{-1}; one unit of SOD activity was defined as the quantity of enzymes necessary to produce 50 % of inhibition on the initial speed of citocrom c reduction in the control reaction at 25ºC (15).

Ascorbate peroxidase activity. This (APX) activity was assayed according to the method described by Hossain and Asada (16), based on ascorbic acid oxidation, which was monitored at 290nm for 1min.

APX activity was determined in 1 mL reaction mixture containing Hepes-NaOH (50 mM pH 7.6), ascorbate (0.2mM), H_2O_2 (0.3mM) and 20-100 μL sample. Reaction was initiated by the addition H_2O_2. The enzymatic activity was expressed as nmoles of oxidized ascorbic acid .min^{-1} and calculated through an extinction coefficient of 2.8 mM^{-1}.cm^{-1} (16).

Statistical analysis. The experiment followed a randomized complete design. Gas exchange and fluorescence results are the means of four independent replicates on different plants of each treatment. SOD and APX activity results are the means of three replicates in each treatment.

The significance of differences between mean values was determined by one-way analysis of variance. Duncan´s multiple range test was used to compare the means when it was necessary. Data presented in the figures are the mean values with their standard error.

RESULTS AND DISCUSION

Gas exchange characteristics and chlorophyll fluorescence. Photosynthetic activity expressed as CO_2 assimilation showed changes with stress conditions. The rate of gas exchange at 45ºC-2h was not significantly reduced in cv Amalia. However, the stressed plants for three hours significantly reduced the rate of gas exchange about 50 %. By contrast, the tolerant type Nagcarlang did not show significant modifications at the rate of gas exchange in stressed plants for both stress conditions (Figure 1).

By the way, stomatal conductance showed different modifications with stress conditions in each genotype. Stomatal conductance presented significant reductions (50 %) in cv Amalia plants under both stress conditions. However, in the tolerant type Nagcarlang, only the stressed plants for three hours presented significant reductions (Figure 2).

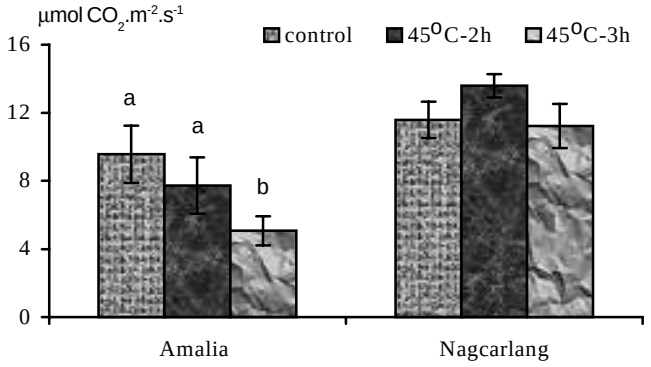

Figure 1. Effect of high temperature on gas exchange in two tomato genotypes (95 % confidence)

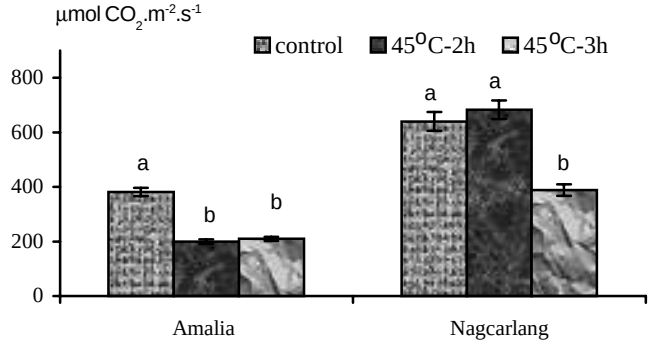

Figure 2. Effect of high temperature on stomatal conductance in two tomato genotypes (95 % confidence)

The reduced values of stomatal conductance observed in stressed plants of cv Amalia for two hours and tolerant type Nagcarlang for three hours did not affect the values of CO_2 assimilation, which indicates the stomatal component did not limit CO_2 diffusion. Specifically, some authors found that net photosynthesis (Pn) depression at high temperature was largely the result of high respiration rates and this depression did not appear to be attributed to increasing stomatal limitations (17). Similar stomatal response to high temperature observed in both cultivars (cv Amalia at two hours and Nagcarlang at three hours) could be associated to tolerance mechanisms to avoid an excessive transpiration maintaining gas exchange values. Cv Amalia reduced gas exchange values at three hours, which indicates some effects on mesophyll component (inhibition of key enzymes).

Previous studies have demonstrated that Rubisco activation in intact leaf tissue was sensitive to rapid increase of leaf temperature (6, 7, 18). Based on what was previously reported (6, 18), it is suggested that heat stress-induced inhibition in CO_2 assimilation can be caused by inhibition of key enzymes on Calvin cycle such as Rubisco (by inhibition of activase), fru-1,6-bisphosphatase (FBPase) and $NADP^+$-dependent glyceraldehydes-3-phosphate dehydrogenase (G3PDH) (by action of free radicals). Changes in activities of Rubisco, other Calvin cycle enzymes, or electron transport with growth temperature have been frequently reported (19).

Chlorophyll fluorescence analysis proved to be a sensitive indicator of heat stress-induced inhibition of gas exchange and initial Rubisco activity (18). High temperature stress and time exposition to stress conditions modified the fluorescence emission in cv Amalia (Figure 3). Fo increases with time exposition to stress conditions. Fo increase with high temperature might be interpreted as a reduction in constant values to trap energy by reaction center of PSII, as a result of physical dissociation of light harvesting complex (1).

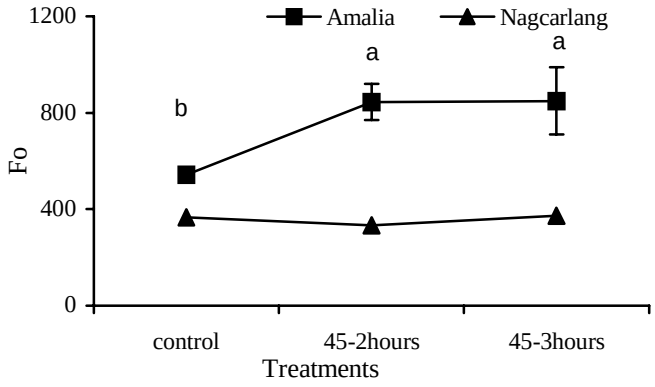

Figure 3. Effect of high temperature on the initial fluorescence (Fo) in two tomato genotypes (95 % confidence)

Photochemical efficiency of PSII expressed as Fv/Fm was reduced by stress conditions in cv Amalia. The tolerant type Nagcarlang did not show modifications in the photosynthetic apparatus with stress conditions (Figure 4).

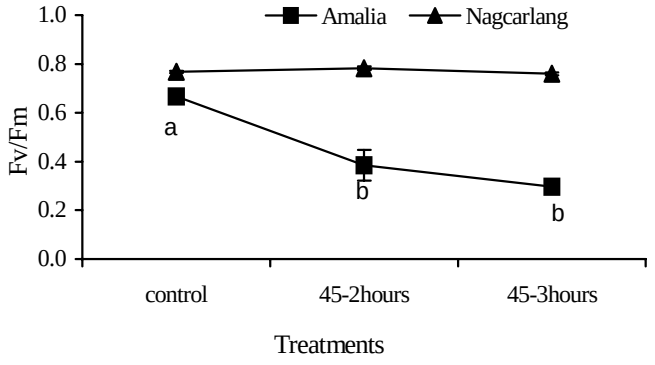

Figure 4. Effect of high temperature on the photochemical efficiency of PSII (Fv/Fm) in tomato genotypes (95 % confidence)

High temperature altered the maximum quantum yield of photochemistry of PSII (Fv/Fm), since two hour exposition, it indicating damage in an important portion of reaction center in PSII, presumably on D1 protein of PSII. It was noted that the effect on the photochemical reaction of PSII appeared before the ones observed in gas exchange, it indicating the vulnerability of PSII to short-

term stress. Inefficient functionality of PSII with high temperature have been found in different species and established that the inactivation of reaction center of PSII is associated to D1 protein degradation (1, 20).

Fluorescence emission and gas exchange were not modified with high temperature in tolerant type Nagcarlang, which indicates that sensitivity/tolerance to heat stress was manifested throughout the photosynthetic apparatus. *Antioxidant activities*. To counteract the toxicity of active oxygen species, a highly efficient antioxidant defense system, including both no enzymatic and enzymatic constituents, is present in plant cells. A relevant defense system on chloroplast is represented by SOD and APX activities, which protect many cellular components against oxidative stress (10, 21). SOD activity decreased by 30 and 70 % in stressed plants of cv Amalia at 45ºC-2h and 45ºC-3h, respectively. Whereas APX activity decreased by 25 and 30 % in stressed plants at 45ºC-2h and 45ºC-3h, respectively. These reduced values in SOD and APX activities suggest some effects in these enzymes, which are moderately resistant to high temperatures (Figure 5 and 6).

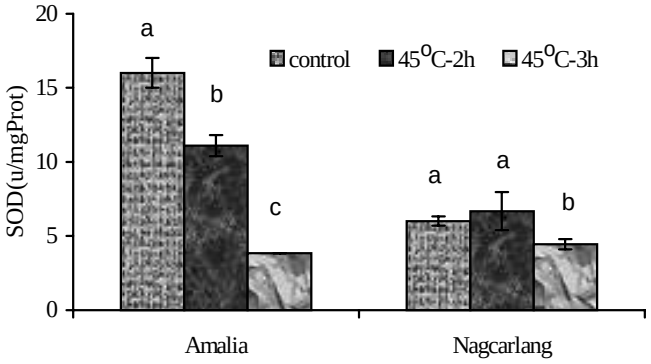

Figure 5. Effect of high temperature on SOD activity in two tomato genotypes (95 % confidence)

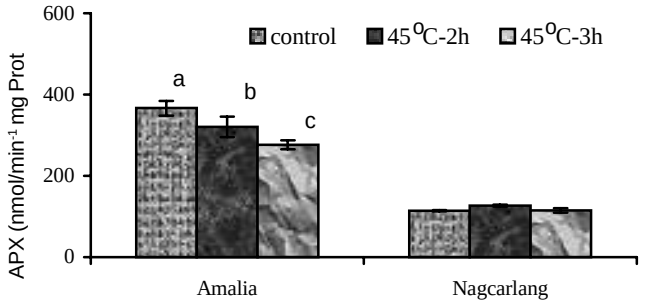

Figure 6. Effect of high temperature on APX activity in two tomato genotypes (95 % confidence)

However, SOD activity decreased in stressed plants at 45ºC-3h, whereas APX activity did not change with stress conditions in tolerant type Nagcarlang (Figures 5 and 6). The high level of antioxidant activities present (in control as well as stressed plants), in cv Amalia was noted, which can be related with its good behavior under nonoptimal conditions.

Antioxidant response was not increased during stress in both genotypes, possibly by the effect of heat stress in SOD and APX enzymes. APX and SOD activities were not related with moderate tolerance and tolerance of the photosynthetic apparatus present in cv Amalia and the type Nagcarlang, respectively. Antioxidant response can be damaged by high temperatures and an increase in the concentration of oxidants can be achieved during stress (22, 23). Accumulation of substances, as hydrogen peroxide, during stress and post-stress, inhibits Calvin cycle enzymes, such as 1,6-bisphosphatase and glyceraldehydes 3-phosphate deshydrogenase which reduces photosynthetic carbon dioxide assimilation (19).

In brief, stress conditions provoked damages to the photosynthetic apparatus in cv Amalia. Its modifications were primarily achieved in reaction center of PSII (since two hour exposition), and later in gas exchange (at three hour exposition). It confirms the moderate thermotolerance of CO_2 assimilation (short-term) capacity and the vulnerability of PSII to high temperatures and that PSII is possibly the primary site of damage during the first hours of stress (two hours). Antioxidant response expressed by SOD and APX activities did not play an important role in defense against oxidative stress neither was it related with tolerance.

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