HIGH TEMPERATURE EFFECT ON TOMATO (*Lycopersicon esculentum*) PIGMENT AND PROTEIN CONTENT AND CELLULAR VIABILITY

Daymi Camejo✉ and W. Torres

ABSTRACT. Tomato cultivars with different sensitivity to high temperature (Campbell-28 and Amalia) and the wild type Nagcarlang, were exposed to different stress conditions (45^0C-two hours of exposition, 45^0C-three hours of exposition and 25^0C as control). "a", "b" and total chlorophylls were determined as well as carotenoid and total soluble protein content, and cellular viability. Chlorophyll "a"/"b" and chlorophyll/carotenoid ratios were estimated. Variables changed depending on cultivars and stress exposure time. Taking Nagcarlang as a model of thermotolerance, variables such as chlorophyll "a"/"b" ratio, chlorophyll/carotenoid ratio, total soluble protein content and cellular viability (TTC assay) might be useful to describe thermotolerant cultivars to high temperature, depending on stress exposure time.

Key words: tomato, *Lycopersicon esculentum*, temperature, pigments, stress, viability, cells

RESUMEN. Cultivares de tomate con diferente sensibilidad a las altas temperaturas (Campbell-28 y Amalia) y el tipo silvestre Nagcarlang, fueron expuestos a diferentes condiciones de estrés (45^oC-dos horas de exposición, 45^oC-tres horas de exposición y 25^oC como control). Los contenidos de clorofila "a", "b" y total fueron determinados, así como los de carotenoides y proteinas totales solubles. La relación clorofila "a"/"b" y clorofila/carotenoides fue estimada a partir de los resultados obtenidos. Las variables evaluadas fueron modificadas según el cultivar y la condición de estrés. La selección de Nagcarlang como modelo de termotolerancia permite establecer que variables tales como: relación de clorofila "a"/"b", clorofila/carotenoides, contenido de proteínas totales solubles y viabilidad celular, pudieran ser útiles para describir cultivares tolerantes a las altas temperaturas, dependiendo del tiempo de exposición al estrés.

Palabras clave: tomate *Lycopersicon esculentum*, temperatura, pigmentos, estrés, vialibidad, células

INTRODUCTION

The recent predictions of global climatic changes, suggest that thermal stress may increase as a result of changes in the thermal environment and alterations in precipitation patterns.

The response of plants to extreme temperatures has been the subject of numerous investigations, much of them emphasized in temperature effects on plant photosynthetic reactions (1, 2). Usually, plants have an optimum temperature for photosynthesis; higher temperatures result in a reduction of photosynthetic rate. It is well known that temperatures above 35ºC generally inhibited photosynthesis of C_3 plants (3).

In tomato plants, high temperatures affect several physiological and biochemical processes dealing finally with yield reduction. Different results pointed to a PS II dysfunction in chloroplasts (2) conducting to a reduction in CO_2 assimilation. Nevertheless, cultivars behave in a differentiated manner under this stressful condition, due to its antioxidant systems (3) and to the synthesis of HSP (4).

Possible biochemical and/or physiological processes affected by temperature are photosynthetic enzyme activity, membrane integrity, photophosphorylation and electron transport in the chloroplast, stomatal conductance to CO_2 diffusion and photoassimilate translocation (5).

Frequently, high temperature produces changes in pigment content of light-harvesting chlorophyll-carotenoid antenna complexes of photosystem I (PS I) and II (PS II) and changes the relation of membrane lipids, that plays an important role preventing membrane photoreduction. When plants are exposed to low or high temperature stress, chlorophyll biosynthesis is affected. Biosynthesis of porphyrins and particularly the chlorophyll "a" during early greening stages of seedlings has been elucidated in detail (6, 7, 8).

The triphenyl tetrazolium chloride (TTC) viability assay has been previously used to study the basic thermal responses of different plants (9) and to demonstrate the role of heat-shock proteins in plant thermotolerance (10). Modification of TTC reduction by temperature is considered to be indicative of the cellular and tissue response to temperature and, therefore, should be affected by induced thermotolerance.

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The use of biological criteria for plant selection is preferred because they are more objective and supply more information about the system (11) and also contribute to the knowledge for a better adaptation to stress conditions.

The experiment was conducted in order to evaluate the effect of high temperature on pigment (chlorophyll and carotenoids) and protein content and cellular viability, in three tomato cultivars with different sensitivity to stress.

MATERIALS AND METHODS

Two tomato cultivars with different sensitivity to high temperature (Campbell-28 (12), susceptible; Amalia (13), with a good behavior, mainly under nonoptimal temperature conditions); and the wild thermotolerant type Nagcarlang (14), were grown under 25/20^{0}C (day/night temperatures) and a 16-hour photoperiod. Plants were grown in pots filled with soil and organic matter (1:3, v/v) and watered daily with nutrient solution.

At the stage of fourth true leaves, plants were exposed to temperature stress (45^{0}C-2h or 45^{o}C-3h). A group maintained untreated (25^{0}C) served as control. Pigment content, total soluble proteins and cellular viability were determined immediately after stress.

For pigment determination a leaf sample (0.25 g) was mashed in a mortar with acetone (80 %, v/v), vacuum filtered to a volumetric flask and completed to volume (25 mL) with acetone. The extract was read at 665, 649, and 652 nm, for chlorophyll "a", chlorophyll "b" and total chlorophyll, respectively; and at 440 nm for total carotenoids.

Total soluble protein content was determined according to Bradford (15) from a chloroplast rich extract obtained from 5 g of leaves. The sample was carefully mashed in the extraction medium containing Mops-KOH (30 mM, pH 7,5), mannitol (350 mM), and cysteine (1 mM) and then centrifuged to 2 200 g for 30 seconds. The extract was read at 595 nm.

Cellular viability was determined by the TTC reduction assay. Three 4.5-mm diameter disks for each treatment were transferred into a tube containing 15 to 20 mL of 0.8 % (w/v) of 2,3,5 triphenyl tetrazolium chloride in phosphate buffer 0.05M to pH=7.4. The discs were vacuum infiltrated with TTC solution by cycles of vacuum on/off about 15 seconds each. Infiltration was considered to be complete when the leaf discs became uniformly colored and sank to the bottom of the tube (9). Tubes were wrapped with foil paper and incubated at 25 ^{0}C in the dark, for 18-20 hours. The rest of the procedure was the same as the one described (9), except that 3 mL of 95 % ethanol was used to extract and dissolve the tetrazolium salt. Absorbance at 485 nm was determined and results expressed as absorbance/total disk area (Abs/m^2).

The experiment was conducted in a completely randomized design. Data presented in the figures and tables are means of three repetitions for viability and six for the other variables. Confidence intervals of treatment means for chlorophylls and carotenoid contents were calculated. Total soluble protein content and cellular viability were analyzed by a completely randomized design; Duncan's multiple range test was used to compare the means when it was necessary.

RESULTS AND DISCUSSION

Chlorophyll "a" content significantly increased in Campbell-28 and Amalia with the exposure at 45^{0}C for three hours, while this increase was shown in Nagcarlang since two hours of exposure to stress (Figure 1).

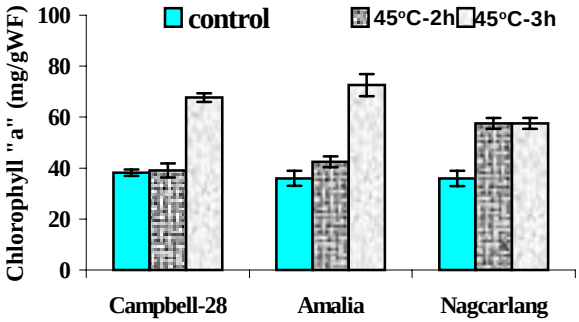

Figure 1. Effect of high temperature on chlorophyll "a" content in three tomato cultivars

Plants exposed to both stress conditions increased chlorophyll "b" content in Campbell-28 (Figure 2), whereas in Amalia the stress condition decreased chlorophyll "b", while a steady decrease was shown with two and three hours in Nagcarlang (Figure 2).

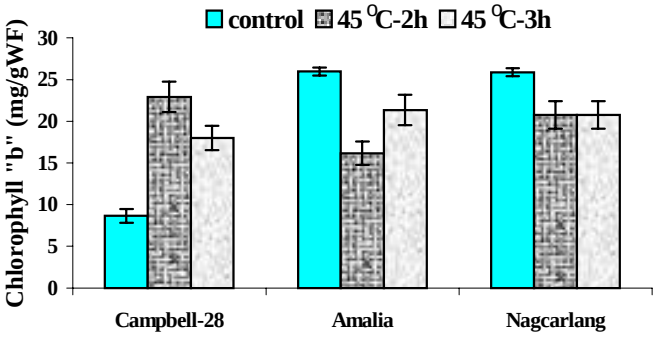

Figure 2. Effect of high temperature on chlorophyll "b" content in three tomato cultivars

Changes in chlorophyll "a" and "b" in stressed plants, led to a total chlorophyll increase in Campbell-28; however, the total chlorophyll content decreased in stressed plants by two hours in cv. Amalia (Figure 3).

This increase in chlorophyll "a" content with stress condition in all cultivars could be associated with a major synthesis and stability of this pigment and a possible adaptation of light-harvesting complexes to stress condition, where the thermotolerant type Nagcarlang exhibited increased contents since two hours of exposition. All varieties exhibited the higher chlorophyll content to

45^{0}C-three hours of exposition, indicating a great emphasis on chlorophyll "a" content, pigment indispensable to photosynthesis. Other authors have found an increase in chlorophyll "a" content in lemon stressed plants (*Eureka)* with high temperatures (16).

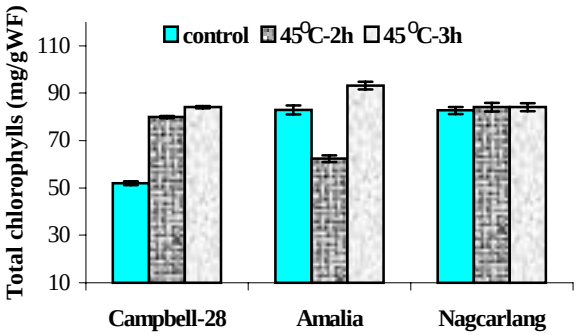

Figure 3. Effect of high temperature on total chlorophyll content in three tomato cultivars

Chlorophyll "a"/"b" ratio increased with heat stress in both, Amalia and Nagcarlang cultivars, while the relation decreased in Campbell-28 with two hours of stress (Figure 4). The increase in chlorophyll "a"/"b" ratio might be associated with the protection of photosynthetic system under stress conditions, due to a lesser radiation absorption at shorter wave length and a reinforcement of PS I functioning (17).

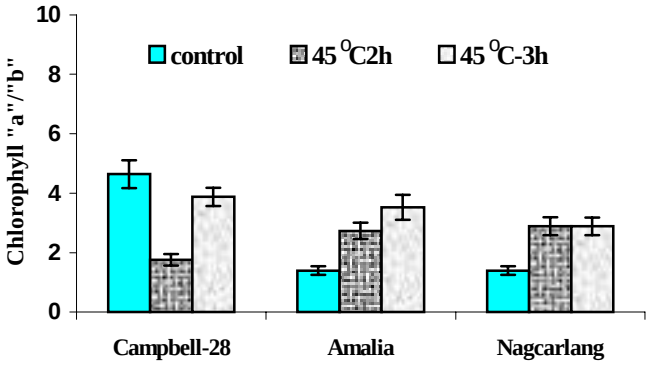

Figure 4. Effect of high temperature on chlorophyll "a"/"b" ratio in three tomato cultivars

Total carotenoid content did not change with stress conditions in cv. Amalia, while increased in Nagcarlang. Campbell-28 showed a decrease in total carotenoid content with two hours of stress while to three hours the values were similar to control plants (Figure 5).

It is well documented that carotenoids not only play a role as accessory light-harvesting pigments but also protect photosynthetic systems as nonenzymatic antioxidant compounds against reactive oxygen species (18, 19, 20) generated during stress. Therefore, maintaining a higher or invariable level of total carotenoids during a stressful condition, allows the plant to tolerate it. These results are in agreement with previous studies related to plant acclimation to stress (18, 21, 22).

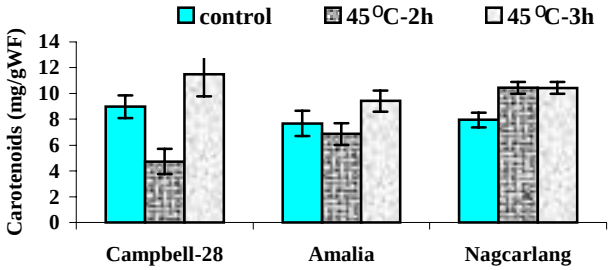

Figure 5. Effect of high temperature on carotenoid content in three tomato cultivars

It is of interest the similarity of the relation chlorophylls/carotenoids in Amalia and Nagcarlang (tolerant type) in both control and stressed plants (Figure 6), indicating that an approximately constant proportion of chlorophylls to carotenoids could be used as tolerance indicator, and a symptom of a better physiological status during stress.

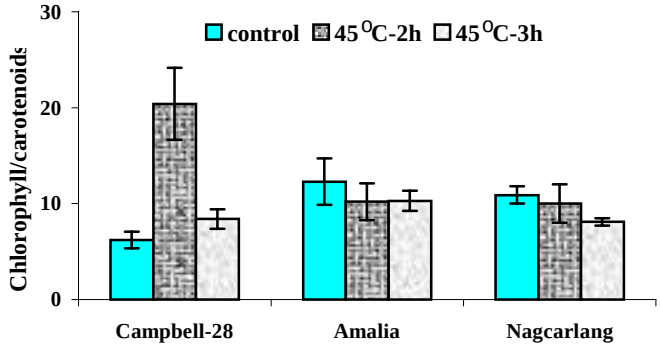

Figure 6. Effect of high temperature on chlorophyll/ carotenoid relation in tomato cultivars

Within the thylakoids, the xanthophylls lutein and neoxanthin are specifically bound to the light-harvesting chlorophyll "a"/"b"-carotenoid-proteins, the LHCPs, whereas ß-carotene is primarily associated with the reaction center chlorophyll "a"/ß-carotene proteins of PS I and II (19). Though, a fixed proportion of these pigments should be maintained for plant normal behavior.

TTC reduction capacity in Campbell-28 did not significantly change with stress condition. By constrast, an increase at 45^{0}C-two hours of exposition in Amalia and the thermotolerant type Nagcarlang was found, suggesting an increase in respiration process and a higher resistance of this enzymatic complex to heat (Table I). Previous studies have shown that the growth respiration is in proportion to the synthesis process and this proportionality is not altered by temperature (23). In this sense Amalia and Nagcarlang increased their synthesis possibility yet with two hours of heat stress.

Table I. Effect of high temperature on TTC reduction in three tomato cultivars

Cultivars	Treatments	TTC reduction (Abs/m^2)
Campbell-28	control	0.35
	45^{0}C-2 hours	0.35
	45^{0}C-3 hours	0.32
		SEx=0.01 ns
Amalia	control	0.32 b
	45^{0}C-2 hours	0.39 a
	45^{0}C-3 hours	0.28 b
		SEx=0.02 **
Nagcarlang	control	0.19 b
	45^{0}C-2 hours	1.37 a
	45^{0}C-3 hours	0.21 b
		SEx=0.04 ***

At 45^{0}C-three hours of exposition, the TTC reduction capacity was similar to control plants, indicating a return to normal conditions.

The TTC reduction assay has been extensively used as a suitable physiological endpoint in plant temperature studies of both intact tissue and plant cell culture (9), and measures enzyme activity, primarily the one associated with mitochondria (24).

When the total soluble protein content was analyzed, a significant increase was found in Amalia and the thermotolerant type Nagcarlang at 45^{0}C-three hours of exposition, while in Campbell-28 the protein content increased with stress but this change was not significant (Table II). It should be noted that Amalia and Nagcarlang had similar response to stress condition; however, the protein content in the former was lower than in thermotolerant type Nagcarlang. The increase in protein content might suggest a change in the gene expression that would be associated with a possible thermotolerance and acclimation to stress condition.

Table II. Effect of high temperature on total protein content in three tomato cultivars

Cultivars	Treatments	Protein content (mg.mL^{-1})
Campbell-28	control	3.76
	45 ^{0}C-2 hours	5.96
	45 ^{0}C-3hours	4.35
		SEx= 0.64 ns
Amalia	control	0.43 b
	45 ^{0}C-2 hours	0.83 b
	45 ^{0}C-3hours	1.98 a
		SEx= 0.15 *
Nagcarlang	control	3.47 c
	45 ^{0}C-2 hours	4.83 b
	45 ^{0}C-3hours	6.10 a
		SEx= 0.01 ***

Several studies report the synthesis of HSP associated with thermotolerance (25, 26); however, little is known about the mechanisms by which they may confer thermotolerance. It has been found that they can function as ATP-independent chaperones (25, 26). *In vitro* studies suggest that HSP prevent the aggregation of substrate proteins caused by heat or chemical denaturation and facilitate the reactivation of denatured substrate proteins. The increase in protein content to elevate temperature observed in Amalia and Nagcarlang could play an important protective role.

In summary, the results presented in this report demonstrate the biochemical changes provoked by high temperature and exposure time in tomato cultivars, related with their susceptibility to stress. Taking Nagcarlang as a model of thermotolerance, variables such as chlorophyll "a"/"b" ratio, chlorophyll/carotenoid ratio, total soluble protein content and cellular sensitivity (TTC assay) might be useful to describe thermotolerant cultivars to high temperature, depending on the exposure time to stress.

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