Indian J. Plant Physiol., Vol. 17, No. 2, (N.S.) pp. 103-112 (April-June, 2012)

HEAT-STRESS EFFECTS ON DRY MATTER PARTITIONING, POLLEN VIABILITY AND FRUIT YIELD IN TOMATO GENOTYPES

ISHWAR SINGH* AND MARIKO SHONO

Japan International Research Center for Agricultural Sciences, Tropical Agriculture Research Front, 1091-1, Maezato-Kawarabaru, Ishigaki, Okinawa 907-002, Japan

Received on 15th November, 2011, Revised and accepted on 20th April, 2012

SUMMARY

Heat-stress is one of the most important limiting factors for crop productivity under sub-tropical conditions, mainly because of its adverse effects on photosynthetic efficiency and pollen fertility. However, induction of heat shock proteins during heat-stress helps the plant to tolerate high temperature. In the present study, we evaluated four tomato genotypes *viz*. CLN 2026E, UC 204A, Suncherry extra and Ailsa Craig for their heat-stress response at different growth stages. The mitochondrial small heat shock proteins (MT-sHSPs) were expressed in leaves of all the four genotypes when the plants were exposed to 38°C for 4 hours. The flowers of CLN 2026E and Suncherry extra started accumulating MT-sHSP within one hour of exposure to 38°C, however, we observed expression of MT-sHSP in CLN 2026E flowers under heat-stress (35/27°C, day/night temperatures) conditions. The genotypes CLN 2026E and Ailsa Craig had significantly higher cell membrane thermostability at high temperature. These genotypes also showed comparatively less reduction in fruit yield at 35/27°C day-night temperatures. Significant reduction in pollen fertility of genotypes UC204A and Suncherry extra was observed during heat-stress. These genotypes did not show *in vitro* pollen germination at 35°C, indicating their sensitivity to high temperature. On the other hand pollen of genotypes CLN 2026E and Ailsa Craig germinated well at 35°C and also even at 40°C little germination was observed, providing the evidence of thermotolerance.

Key words: **Cell membrane thermostability, dry matter partitioning, heat-stress, mitochondrial sHSP, pollen fertility, pollen germination**

Abbreviations: DAS: Days after sowing, MT-sHSP: Mitochondrial small heat shock protein

INTRODUCTION

High temperature stress is one of the most important limiting factors for crop productivity, besides drought and salinity, under sub-tropical conditions. Inhibition of growth under supra-optimal temperature can result from thermal effects on many physiological and developmental processes. Photosynthesis, in particular is one of the most heat-sensitive functions of the plant cell (Camejo *et al*. 2005, Singh *et al*. 2005). Temperature in the range of 35-45°C tends to inhibit photosynthesis in C_3 plants. The photosynthetic rate increases with rise in temperature until an optimal range, after which an increase in temperature results in a decrease in the rate of photosynthesis. Among the photosynthetic components, high temperature affects the Calvin cycle, photosystem

*Corresponding author: isingh.dmr@gmail.com, Present address: Directorate of Maize Research, Indian Agricultural Research Institute, Pusa Campus, New Delhi-110 012, India

II activity (Camejo *et al.* 2005), photophosphorylation (Havaux 1992) and Rubisco activity (Karim *et al*. 1999). High thermostabilities of cell membranes and photosynthesis have been reported as an adaptation to high temperature in crop plants (Moffet *et al*. 1990). Genotypic differences in cell membrane thermostability and its correlation with field performance have been reported in soybean (Martineau *et al*. 1979) and cabbage (Chauhan and Senboku 1996) and tomato (Camejo *et al*. 2005). Alleviation of heat-stress by the expression of mitochondrial sHSPs has been reported in a number of plant species (Lund *et al.* 1998, Liu and Shono 1999, Shono *et al.* 2002) and it is suggested that MT-sHSPs are involved in heat tolerance of crop plants and play a pivotal role in enhancing thermotolerance (Sanmiya *et al.* 2004, Nautiyal *et al*. 2005, Singh and Shono 2005).

The detrimental effects of heat-stress are more pronounced at reproductive phase. Exposure to high temperature during reproduction caused a reduction in fertility in tomato (Song *et al*. 1999, Pressman *et al*. 2002, Singh and Shono 2003), bean (Weaver *et al*. 1985), tobacco (Shivana *et al*. 1991), *Brassica* (Rao *et al*. 1992) and Pigeonpea (Singh *et al*. 1992) due to failure of pollination and fertilization. Flowering and fruit setting are highly heat sensitive processes of tomato plant and relate directly to yield. Among the essential processes responsible for fruit set, production of viable pollen, pollen germination on the stigma and pollen tube growth down the style are particularly sensitive to high temperature (Singh *et al*. 1992, Suzuki *et al*. 1999). Failure of normal endothecium formation, excessive elongation of style above the antheridial cone before anthesis, ovule damage and alterations in carbohydrate metabolism in developing anther (Pressman *et al.* 2002) also account for poor fruit setting under high temperature conditions. High temperature is also reported to affect membrane integrity of pollen tube (Shivana and Cresti 1989). In the present study we evaluated four tomato genotypes for their heat-stress response at different growth stages.

MATERIAL AND METHODS

*Plant material***:** Four tomato (*Solanum lycopersicum*) genotypes, viz. Ailsa Craig, Suncherry extra, CLN 2026E* and UC 204A* (*seed material was procured from AVRDC, Taiwan) were used in this study. Seedlings were raised in small plastic pots (9 cm top diameter) filled with 200 g sand, vermiculite and FYM (2:1:1) with five seeds per pot in a green house under natural daylight and 25/20°C day-night temperatures. When the seedlings were three weeks old, they were transplanted to large waghner plastic pots (16 cm top diameter). Two seedlings per pot were transplanted, while upon establishment were thinned to one per pot. The plants were irrigated daily and 2 g of composite fertilizer (containing N,P,K in the ratio of 14:14:14) was applied to each plant at fortnight interval. The pots were arranged in completely randomized design in two sets and kept in a green house maintained at 25/20°C day-night temperatures (control) until initiation of flowering (60 DAS) under natural illumination. Each set consisted of five pots. One set of pots was then moved to another green house maintained at 35/27°C (heat-stress) day-night temperatures.

Protein analysis: Samples of leaf and flower for analysis of MT-sHSP were collected from both green houses after two weeks of temperature treatment. The proteins were extracted from composite sample of leaf/flower tissues (500 mg) and analyzed by western blotting. The leaf/flower tissues were macerated separately in 3 ml lysis buffer consisting of 0.25 M sucrose, 20 mM Tris-HCl (pH 7.5), 0.5 mM ethylene diamine tetra acetic acid (EDTA) and 2.0% w/v polyvinyl pyrrolidine (PVP) in ice cooled pestle mortar. The homogenate was centrifuged at 1000xg for 10 min at 4°C. The pellet was discarded and the supernatant was again centrifuged at 10,000xg for 30 min at 4°C. The pellet was dissolved in 50 µl extraction buffer (20 mM Tris-HCl, pH 7.5+0.5 mM EDTA). The samples were prepared by mixing 50 µl sample with 50 µl sample buffer (consisting of 60 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS and 0.002% bromophenol blue). Just before use the sample buffer was mixed with 2 mercaptoethanol in a ratio of 9:1.

For western blot analysis, proteins (5 µl of each sample) were separated on SDS-PAGE according to the method of Laemmli (1970) and transferred on to PVDF membrane by electroblotting using the wet type

electroblotting cell (Model NA-1510, Nippon Eido, Japan). The MT-sHSPs were detected by sequential incubation with anti-MT-sHSPs antibodies and peroxidase conjugated anti-rabbit IgG antiserum, each at a dilution of 1:1000, followed by chromogenic visualization by a peroxidase Kit (Vector Laboratories Inc., Burlingame, California, USA). The anti-MT-sHSP antibody was produced as per the procedure described by Sanmiya *et al*. (2004). The full-length cDNA for the tomato MT-sHSP gene was sub-cloned into the glutathione S-tranferase (GST) vector (Amersham), an expression vector in *E coli.* The GST-MT-sHSP fusion protein was purified according to instruction manual and used as the antigen. Rabbit anti-MT-sHSP antibody was produced and affinity-purified by Sawady technology. Each experiment was repeated three times and the patterns of MT-sHSP accumulation were found to be consistently reproducible.

Cell membrane thermostability: Cell membrane thermostability of leaf tissues was measured according to method described by Martineau *et al*. (1979) after two weeks of temperature treatment. Each sample for assay consisted of a paired set (treatment and control) of 20 leaf discs (1.0 cm diameter) cut from two young leaves. Before an assay the paired set of leaf discs was placed into two separate tubes and washed thoroughly with distilled water with at least three change of water, in order to remove endogenous electrolytes from cut ends of leaf discs. After final wash excess water was drained off. The treatment tubes were then covered with plastic wrap and incubated in thermostatically controlled water baths for 15 min at 30, 35, 40 and 45°C. The control tubes were maintained at 25°C for the same period. The treatment tubes were then allowed to cool down to 25°C and poured with 25 ml distilled water (also in control tubes). Both control and treatment tubes were incubated at 10°C overnight (12-18 h) to facilitate the diffusion of electrolytes from the discs. The tubes were then brought to room temperature (25°C), inverted several times to mix the contents and initial conductance was measured using an electrical conductivity meter (CM-115, Kyoto Electronics, Japan). After completing the measurements, tubes were covered with aluminium foil and autoclaved at 120°C for 10 min to completely kill the leaf tissues. The tubes were then cooled down to 25°C and contents were mixed thoroughly and final conductance was measured. The degree of relative injury as a result of temperature treatment was calculated as follows.

$$\text{Relative Injury (\%)} = [1- (T_i / T_f) / (1- (C_i / C_f)] \times 100$$

Where 'T' and 'C' refer to conductance values for treatment and control tubes, respectively and the subscripts 'i' and 'f' to initial and final conductance, respectively.

Pollen fertility and germination: Pollen grains were collected on butter paper between 9:00 to 11:00 h from freshly opened flowers from both the green houses after 3-4 weeks of temperature treatment and inoculated on germinating medium as described by Singh and Shono (2003) consisting of 15% sucrose (w/v), 250 mg l^{-1} boric acid, 200 mg l^{-1} calcium nitrate and 0.8% agar. The germinating mixture was boiled for five minutes in a microwave oven and allowed to cool at a temperature till the medium started solidifying. EBR at final concentrations of 1 µM, 10 µM and 50 µM was added quickly to the basal medium, 5 ml was poured in each petri-plate (6 cm diameter) and they were allowed to cool at room temperature (25 ± 2°C). These were then inoculated with pollen and placed in incubators maintained at 25, 35 and 40°C. After 4 h of incubation, the petriplates were taken out and in each plate 2 ml staining (safranin 0.02% w/v), killing and fixing solution (glycerol, formaldehyde, glacial acetic acid and distilled water, 20:5:3:72) was poured. The petriplates were examined under phase contrast microscope (100x). Ten readings per plate for pollen germination and pollen tube length were taken by recording on microscopic field basis.

Pollen fertility was determined by pollen stainability with acetocarmine (Suzuki *et al*. 1999) from anthers collected from freshly opened flowers and fixed in acetic acid: ethanol (3:1).

Dry matter partitioning: Four months old fully mature plants were harvested from both the green houses. Each plant was separated into different plant parts *viz*. Roots, stem and leaves. The roots were washed thoroughly with tap water to remove the soil particles and then allowed

to dry over night at room temperature. The plant parts were weighed separately and then dried in a temperature controlled oven maintained at 80 ± 2°C for 48 hours followed by measurement of dry weight.

Yield and its attributes: Fruits were harvested twice a week from both the green houses and data were recorded for fruit weight, number and size. After final harvest at about 4 months old plants, the data were pooled to get fruit weight and number per plant and averaged to get mean values. The data were analyzed statistically and difference among heat-stress and control were evaluated by the least significant differences ($LSD_{0.05}$).

RESULTS

Expression of MT-sHSP during heat-stress: The MT-sHSPs were expressed in leaves of all the genotypes, when four weeks old seedlings were exposed to 38°C for 4 h (Fig. 1). The flowers of CLN 2026E and Suncherry extra (only two genotypes were used in this experiment) started accumulating MT-sHSP within one h of heat treatment (Fig. 3). Western blotting analysis of flowers collected from two green houses revealed expression of MT-sHSP in CLN 2026E flowers from high temperature green house (Fig. 2), indicating basic thermotolerance in this genotype.

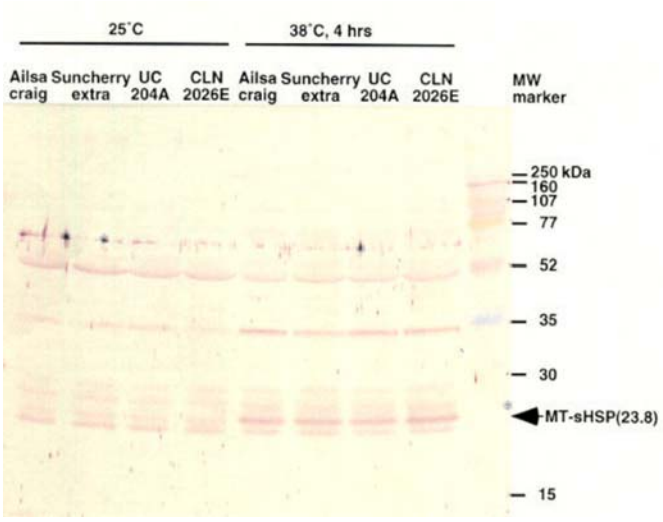

Fig. 1. Heat-stress induced expression of MT-sHSP in tomato leaves. Four weeks old tomato seedlings were treated at 25 and 38°C for 4h prior to sample collection.

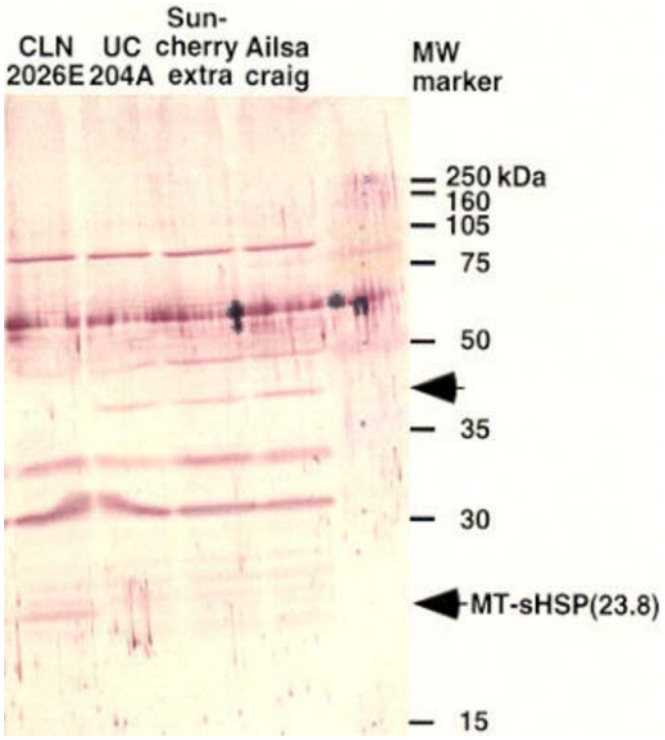

Fig. 2. Heat-stress induced expression of MT-sHSP in tomato flowers. Flower samples for protein analysis were collected from control (25/20°C, day-night temperatures) and heat-stressed (35/27°C, day-night temperatures) green houses after two weeks of temperature treatment.

Effect of heat-stress on thermostability of cell membrane: Genotypes Ailsa Craig and CLN 2026E had significantly higher cell membrane thermostabilities of leaf tissues collected from plants growing at 25/20°C day-night temperatures (Fig. 4). The relative injury increased with rise in temperature in all the four genotypes. However, the increase was significantly higher in UC 204A and Suncherry extra.

Effect of heat-stress on pollen fertility and germination: The pollen fertility did not differ so much among the four genotypes under normal green house conditions (25/20°C) but reduced significantly at high temperature (35/27°C), however, the magnitude of reduction in fertility was much higher in genotypes UC 204A and Suncherry extra (Fig. 5). *In vitro* studies on germination of pollen collected from normal green house revealed higher percent pollen germination in Suncherry extra and Ailsa Craig at 25°C and were related with tube

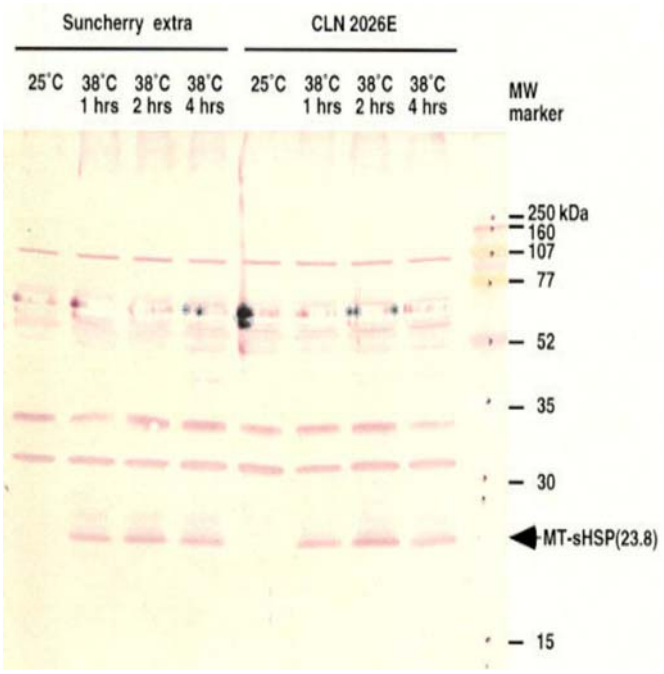

Fig. 3. Time sequence of MT-sHSP expression in tomato flowers. Tomato plants were treated at 38°C in growth chamber and flower samples for protein analysis were collected at 0, 1, 2 and 4 hours after temperature treatment.

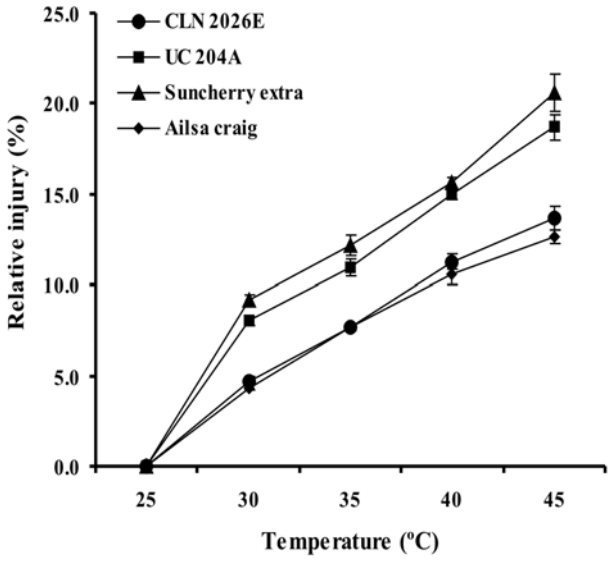

Fig. 4. Relationship between the degree of temperature induced relative injury of leaf tissues and the 15 minutes injury induction temperature in tomato genotypes. Each value is the mean ± SEm of three replicates.

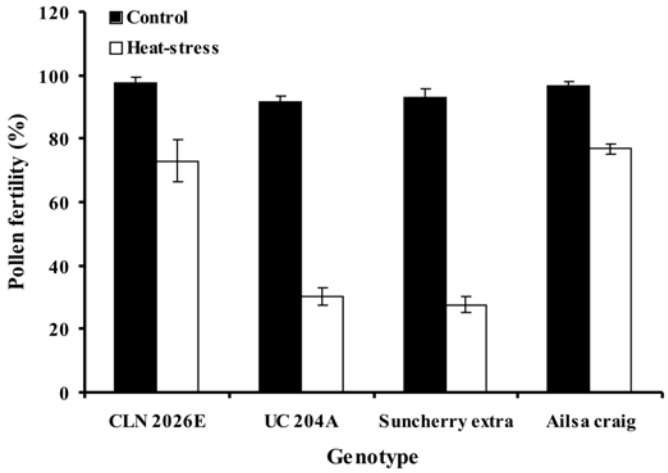

Fig. 5. Effect of heat-stress on pollen fertility in tomato genotypes. Each value is the mean ± SEm of five replicates. Pollens were collected from tomato plants grown under normal (C, 25/20°C day-night temperatures) and heat-stressed (HS, 35/27°C day-night temperatures) green houses.

length. Pollen germination decreased sharply in genotypes, UC 204A and Suncherry extra with corresponding decrease in tube length at 35°C (Table 1). No pollen germination was observed at 40°C in these genotypes (Table 1). Heat-stress imposed during flowering stage adversely affected *in vitro* pollen germination. Significant reduction in germination and tube length was observed in pollen collected from heat-stressed plants (Table 1). The pollen grains of UC 204A and Suncherry extra failed to germinate even at 35°C. However, pollen of Ailsa Craig and CLN 2026E germinated well at 35°C and also showed some germination even at 40°C (Table 1).

Effect of heat-stress on dry matter partitioning, fruit yield and its attributes: Dry matter-partitioning play an important role in economic yield of crop plants. Heat-stress during different growth stages, adversely affects plant productivity. We studied the influence of heat-stress imposed at the onset of flowering stage on dry matter distribution in different plant parts at the time of harvest (120 DAS). A decrease in fresh weight with corresponding decrease in dry weight of leaf, stem and roots was recorded under heat stressed conditions in genotypes, Ailsa Craig and CLN 2026E. Roots of these genotypes showed maximum reduction in fresh and dry

Table 1. Effect of incubation temperature on *in vitro* pollen germination (%) and pollen tube length (µm) in tomato genotypes under normal (C) and heat-stress (HS) conditions. Each value is the mean of ten readings ± SEm (n=10).

Genotype	Treatment	Incubation temperature (ºC)		
		25	35	40
Pollen germination (%)				
CLN 2026E	C	67.7 ± 4.5	30.4 ± 5.0	7.2 ± 1.1
	HS	48.6 ± 7.3	25.2 ± 1.9	4.3 ± 1.1
Ailsa Craig	C	89.9 ± 5.4	70.1 ± 4.3	7.7 ± 1.3
	HS	83.0 ± 1.5	52.0 ± 5.5	4.0 ± 0.7
UC 204A	C	46.4 ± 6.1	3.9 ± 1.3	0.0 ± 0.0
	HS	29.9 ± 3.9	0.0 ± 0.0	0.0 ± 0.0
Suncherry extra	C	85.6 ± 2.1	7.0 ± 1.6	0.0 ± 0.0
	HS	22.7 ± 2.2	0.0 ± 0.0	0.0 ± 0.0
Pollen tube length (µm)				
CLN 2026E	C	849.7 ± 60.6	241.6 ± 25.5	45.9 ± 5.4
	HS	269.3 ± 14.1	147.9 ± 15.5	27.4 ± 1.4
Ailsa Craig	C	1134.2 ± 84.6	418.2 ± 17.2	49.9 ± 7.1
	HS	928.2 ± 36.1	312.1 ± 21.7	26.5 ± 1.1
UC 204A	C	960.8 ± 54.7	74.5 ± 1.6	0.0 ± 0.0
	HS	458.0 ± 17.4	0.0 ± 0.0	0.0 ± 0.0
Suncherry extra	C	1037.3 ± 57.9	258.1 ± 11.2	0.0 ± 0.0
	HS	515.1 ± 12.5	0.0 ± 0.0	0.0 ± 0.0

Pollens were collected from tomato plants grown under normal (C, 25/20°C day-night temperatures) and heat-stressed (HS, 35/27°C day-night temperatures) green houses.

weights by heat-stress (Table 2). On the other hand heat-stress enhanced both fresh and dry weights of leaf, stem and roots of genotypes UC204A and Suncherry extra (Table 2). In these genotypes we noticed maximum increase of fresh and dry weights in stem. Heat-stress also decreased fruit yield and this decrease was almost double in UC 204A and Suncherry extra. Interestingly, when we pooled the fresh weight of leaf, stem, roots and fruits together, no significant reduction was observed in genotypes UC 204A and Suncherry extra, which otherwise decreased significantly in CLN 2026E and Ailsa Craig. We presume that under high temperature stress, the dry matter produced in genotypes UC 204A and Suncherry extra was diverted towards the vegetative instead of reproductive parts and thus possibly caused reduction in yield.

Maximum yield per plant was recorded in Ailsa Craig (497.28 g) and CLN 2026E-(450.44 g) under heat-stress. The mean fruit weight was maximum in UC 204A (38.94 g) and minimum in Suncherry extra (7.71 g) and was correlated positively with fruit size and negatively with fruit number (Table 3). The heat-stress enhanced mean fruit weight in genotypes CLN 2026E and Ailsa Craig, however, fruit size was decreased by heat-stress in all the four genotypes.

DISCUSSION

Heat-stress is one of the most important limiting factors for crop productivity under sub-tropical conditions, mainly because of its adverse effects on photosynthetic efficiency and pollen fertility. However, induction of heat shock proteins during high temperature stress is reported to help the plant to tolerate high temperature (Schlesinger 1990, Vieling 1991, Sanmiya *et al*. 2004, Neta-Sharir *et al.* 2005). We observed the expression of MT-sHSP in tomato leaves and flowers, when whole plants were treated at 38°C for 4 and 2 hours respectively (Fig. 1, 3). Lund and Elthion (1998)

also reported alleviation of heat stress in maize by the expression of MT-sHSPs in leaves under field conditions. However, there is no report available in literature about heat induction of MT-sHSP in flower, which seems to be important in plant reproduction.

Higher thermostabilities of cell membranes and photosynthesis have been reported to contribute to the adaptation to high temperature in crop plants (Moffet *et al*. 1990, Camejo *et al.* 2005). Genotypic differences in cell membrane thermostability and its correlation with field performance have been reported in soybean (Martineau *et al*. 1979), cabbage (Chauhan and Senboku 1996) and maize (Coskun *et al*. 2011). The genotypes Ailsa Craig and CLN 2026E showed significantly higher thermostabilities of leaf tissues (Fig. 4), which was related with fruit yield (Table 4). The high temperature induced injury to leaf- tissues, enhance membrane permeability, which allow the electrolytes to diffuse out of the cell (Martineau *et al*. 1979). Thus thermostability of the cell membrane depends on the degree of electrolyte leakage. The membrane disruption caused by

Table 2. Effect of heat-stress on fresh and dry weight of leaf, stem and roots of four tomato genotypes. Each value is the mean of three independent plants. Values in parentheses represent the percent increase (+) or decrease (-) over the control.

Genotype	Treatment	Fresh weight (g)				Dry weight (g)			
		Leaf	Stem	Roots	Total	Leaf	Stem	Roots	Total
Ailsa Craig	C	197.0	208.3	29.3	428.6	43.7	52.8	4.9	101.4
	HS	169.3	201.0	24.8	395.1(-7.8)	39.4	51.7	4.1	95.2(-6.0)
CLN 2026E	C	210.7	134.0	49.2	397.9	40.6	34.0	8.3	82.9
	HS	168.7	120.7	35.2	324.6(-18.4)	36.2	29.2	5.0	70.4(-15.0)
UC 204A	C	251.7	98.7	47.6	398.0	50.8	30.1	8.4	89.3
	HS	282.7	155.0	66.8	504.5(+26.8)	60.4	40.3	9.3	110.0(+23.3)
Suncherry extra	C	147.0	212.7	24.8	384.5	37.0	52.3	4.4	93.7
	HS	153.0	278.0	25.3	456.3(+18.7)	43.2	65.1	4.0	112.3(+19.7)

Tomato plants were grown under normal (C, 25/20°C day-night temperatures) and heat stressed (HS, 35/27°C day-night temperatures) green houses.

Table 3. Effect of heat-stress on yield and its attributes in tomato genotypes. Each value is the mean of three independent plants.

Genotype	Treatment	Fruit weight plant^{-1} (g)	Fruit number	Mean fruit weight (g)	Fruit size (cm)
Ailsa Craig	C	547.5	16.3	33.5	4.2
	HS	450.4	12.7	35.6	4.1
CLN 2026E	C	601.0	22.7	26.5	4.2
	HS	497.3	16.0	31.1	4.1
UC 204A	C	324.4	8.3	38.9	4.6
	HS	203.1	6.0	33.9	4.2
Suncherry extra	C	228.8	29.7	7.7	2.7
	HS	139.0	20.3	6.8	2.2
$LSD_{0.05}$		37.0	2.4	1.8	0.3

Tomato plants were grown under normal (C, 25/20°C day-night temperatures) and heat stressed (HS, 35/27°C day-night temperatures) green houses.

high temperature may alter water ion and organic solute movement, photosynthesis and respiration. The membrane thermostability may be associated with changes in the degree of membrane's lipid saturation (Raison *et al*. 1982, Ivanova *et al*. 1993). Murakami *et al*. (2000) had provided best evidence for improving thermotolerance of plants by enhanced thylakoid membrane thermostability, due to increased unsaturated lipids (fatty acids with double bonds). The reduced level of lipid unsaturation improved the rate of photosynthesis and plant growth at high temperature (Gombos *et al.* 1994). The membrane thermostability could be associated with the expression of HSPs in plants under heat-stress (Krishnan *et al.* 1989).

Flowering and fruit setting are highly heat sensitive processes of tomato plant (Dane *et al*. 1991, Singh and Shono, 2003, 2005) and relate directly to yield. We observed significant reduction in pollen fertility (Fig. 5) and germination in tomato genotypes, when heat-stress was imposed at flowering stage, though the magnitude of reduction was less in heat tolerant genotypes than the sensitive one. Pollen viability (Pressman *et al*. 2002, Salem *et al*. 2007) and fertility (Dane *et al*. 1991, Suzuki *et al*. 1999) have been reported to be reliable parameters for better plant productivity during heat stress. Higher pollen fertility and viability in heat tolerant genotypes could be due to enhanced membrane thermostability of anthers and membrane integrity of pollen tube (Shivana and Cresti 1989). Wolters-Arts *et al*. (1998) reported involvement of lipids in directional pollen tube growth and it seems that desaturation of lipids in pollen tube is responsible for higher pollen germination and tube length in heat tolerant genotypes. Pressman *et al*. (2002) suggested that the effect of heat-stress on pollen viability was associated with the carbohydrate metabolism in various parts of anther during its development. The heat-stress induced decrease in the sugar concentration in pollen grains is possibly responsible for decreased pollen viability. Synthesis of low molecular weight heat shock proteins and their mRNA has been reported in pollen of *Nicotiana tabacum* (Shono *et al*. 2002). It is possible that these HSPs play a role in the tolerance of pollen grains to high temperature stress. The heat-stress induced diversion of dry matter towards vegetative (stem, roots) instead of reproductive parts in heat sensitive genotypes (Table 2) and this could be the possible reason for higher yield reduction in these genotypes. Turner and Wein (1994) also reported similar findings in pepper. The high temperature stress caused less reduction in fruit yield in tolerant genotypes (Ailsa Craig, CLN 2026E), which had higher mean fruit weight under heat-stress (Table 3). MT-sHSP seems to be of overall advantage to tomato productivity during heat stress.

ACKNOWLEDGEMENT

The senior author is thankful to the Director-General, JIRCAS, Japan for awarding visiting research fellowship. This research was supported in part by funds from the Bio-oriented Technology Research Advancement Institution, Japan.

REFERENCES

Camejo, D., Rodrigues, P., Morales, M.A., Dell'Amico, J.M., Torr ecillas, A. and Alarcon, J.J. (2005). High temperature effets on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.* **162:** 281-289.

Chauhan, Y.S. and Senboku, T. (1996). Thermostabilities of cell membrane and photosynthesis in cabbage cultivars differing in heat tolerance. *J. Plant Physiol.* **149:** 729-734.

Coskun, Y., Coskun, A., Demirel, U. and Odzen, M. (2011). Physiological response of maize (*Zea mays* L.) to high temperature stress. *Aust. J. Crop Sci*. **5:** 966-972.

Dane, F., Hunter, A.G. and Chambliss, O.L. (1991). Fruit set, pollen fertility, and combining ability of selected tomato genotypes under high temperature field conditions. *J. Amer. Hort. Sci*. **116:** 906-910.

Gombos, Z., Wada, H., Hideg, E. and Mrata, N. (1994). The unsaturation of membrane lipids stabilizes photosynthesis against heat stress. *Plant Physiol*. **104:** 563-567.

Havaux, M. (1992). Stress tolerance of photosystem II *in vivo*. Antagonistic effects of water, heat and photoinhibition stresses. *Plant Physiol.* **100:** 424-432.

Ivanova, A.P., Yordanov, I.T., Stefenov, K.L., Seizova, K.A.

and Popov, S.S. (1993). Effect of heat stress on the lipid and fatty acid Composition of the thylakoid membranes in two cultivars of pea acclimated to elevated temperatures. *Photosynthetica.* **29:** 131-137.

Karim, M.A., Fracheboud, Y. and Stamp, P. (1999). Photosynthetic activity of developing leaves of *Zea mays* is less affected by heat stress than that of developed leaves. *Physiol. Plant.* **105:** 685-693.

Krishnan, M., Nguyen, H.T. and Burke, J.J. (1989). Heat shock protein synthesis and thermal tolerance in wheat. *Plant Physiol.* **90**: 140-145.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophase T4. *Nature.* **227:** 680-685.

Liu, J. and Shono, M. (1999). Characterization of mitochondria-located small heat shock protein from tomato (*Lycopersicon esculentum*). *Plant Cell Physiol.* **40:** 1297-1304.

Lund, A.A. and Elthon, T.E. (1998). Changes in maize respiration and mitochondrial function resulting from heat stress under controlled and field conditions. In: I.M. Moller, P. Gardestrom, K. Glimelius and E. Glaser (eds.), Plant Mitochondria: From Gene to Function, pp 335-341. Backhuys Publishers, Leiden, The Netherlands.

Lund, A.A., Blum, P.H., Bhattamakki, D. and Elthon, T.E. (1998). Heat- stress response of maize mitochondria. *Plant Physiol.* **116:** 1097-1110.

Martineau, J.R., Specht, J.E., Williams, J.H. and Sullivan, C.Y. (1979). Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. *Crop Sci.* **19:** 75-78.

Moffet, J.M., Sears, R.G. and Paulsen, G.M. (1990). Wheat high temperature tolerance during reproductive growth. I. Evaluation by chlorophyll fluorescence. *Crop Sci.* **30:** 881-885.

Murakami, Y., Tsuyama, M., Kobayashi, Y., Kodama, H. and Iba, K. (2000). Trienoic fatty acids and plant tolerance of high temperature. *Sci.* **287:** 476-479.

Nautiyal, P.C., Shono, M. and Egawa, Y. (2005). Enhanced thermotolerance of the vegetative part of MT-sHSP transgenic tomato line. *Scientia Hort.* **105:** 393-409.

Neta-Sharir, I., Isaacson, T., Lurie, S. and Weiss, D. (2005). Dual role of tomato heat shock protein 21: Protecting photosystem II from oxidative stress and promoting color changes during fruit maturation. *The Plant Cell*. **17:** 1829-1838.

Pressman, E., Peet, M.M. and Pharr, D.M. (2002). The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. *Ann. Bot.* **90:** 631-636.

Raison, J.K., Roberts, J.K.M. and Berry, J.A. (1982). Correlation between the thermal stability of chloroplast (thylakoid) membrane and the Composition and fluidity of their polar lipids upon acclimation of the higher plant Nerum oleander to growth temperature. *Biochim Biophys. Acta*. **688:** 218-228.

Rao, G.U., Jain, A. and Shivana, K.R. (1992). Effect of high temperature stress on *Brassica* pollen: viability, germination and ability to set fruits and seeds. *Ann. Bot.* **68:** 193-198.

Salem, M.A., Kakani, V.G., Koti, S. and Reddy, K.R. (2007). Pollen-based screening of soybean genotypes for high temperatures. *Crop Sci.* **47:** 219–231.

Sanmiya, K., Suzuki, K., Egawa Y. and Shono M. (2004). Mitochondrial small heat-shock protein enhances thermotolerance in tobacco plants. *FEBS Lett.* **557:** 265-268.

Schlesinger, M.J. (1990). Heat shock proteins. *J. Biol. Chem.* **265:** 12111-12114.

Shivana, K.R. and Cresti, M. (1989). Effect of high humidity and temperature stress on pollen membrane integrity and pollen vigour in *Nicotiana tabacum*. *Sexual Plant Reprod.* **2:** 137-141.

Shivana, K.R., Linskens, H.F. and Cresti, M. (1991). Response of tobacco pollen to high humidity and heat stress: viability and germinability *in vitro* and *in vivo*. *Sex. Plant Reprod.* **4:** 104-109.

Shono, M., Liu, J., Sanmiya, K., Singh, I., Jalaluddin, Suzuki, K., Tsukaguchi, T. and Egawa, Y. (2002). Functional analysis of mitochondrial small heat shock protein. *JIRCAS Working Report.* **23:** 17-23.

Singh, I. and Shono, M. (2003). Effect of 24-epibrassinolide on pollen viability during heat stress in tomato. *Indian J. Exp. Biol.* **41:** 174-176.

Singh, I. and Shono, M. (2005). Physiological and molecular effects of 24-epibrassinolide, a brassinosteroid on thermotolerance of tomato. *Plant Growth Regul.* **47**: 111-119.

Singh, I., Bharti, S., Nandwal, A.S., Goswami, C.L. and Varma, S.K. (1992). Effect of temperature on *in vitro* pollen germination in pigeonpea. *Biol. Plant.* **34:** 461-464.

Singh, I., Shono, M., Fukamachi, H. and Suzuki, K. (2005). Effect of heat stress on gas exchange characteristics in tomato. *Indian J. Plant Physiol.* **10:** 283-286.

Song, J., Nada, K. and Tachibana, S. (1999). Ameliorative effect of polyamines on the high temperature inhibition of *in vitro* pollen germination in tomato (*Lycopersicon esculentum* Mill.). *Scientia Hort.* **80:** 203-212.

Suzuki, K., Takeda, H., Matsuura, S., Yuo, S. and Egawa, Y. (1999). Morphological study on injury of pollen of snap bean by heat stress. Proc. Int. Symp. "World Food Security", Kyoto, Japan. pp. 203-206.

Turner, A.D. and Wein, H.C. (1994). Dry matter assimilation and partitioning in pepper cultivars differing in susceptibility to stress induced bud and flower abscission. *Ann. Bot.* **73:** 617-622.

Vierling, E. (1991). The role of heat shock proteins in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42:** 579-620.

Weaver, M.L., Timm, H., Silbernagel, M.J. and Burke, D.W. (1985). Pollen staining and high temperature tolerance of bean. *J. Amer. Hort. Sci.* **110:** 797-799.

Wolters-Arts, M., Lush, W.M. and Mariani, C. (1998). Lipids are required for directional pollen tube growth. *Nature.* **392:** 818-21.