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Response of Contrasting Tomato Genotypes under High Temperature Stress

A. N. Lokesha, K. S. Shivashankara, R. H. Laxman, A. T. Sadashiva and A. G. Shankar
Department of Crop Physiology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
E-mail: Shivashankara.KS@icar.gov.in

Abstract

The present study was undertaken to evaluate the response of six contrasting tomato genotypes to high temperature stress. Tomato genotypes were grown under polytunnel to expose them to high temperature (40 ± 2°C) for ten days after flower initiation. Physiological parameters *viz.,* relative water content (RWC), epicuticular wax content, membrane stability index, lipid peroxidation (MDA content) and specific leaf area were recorded in the leaf samples collected second, fifth and ninth day after imposition of high temperature treatment. Membrane stability index decreased significantly in IIHR-2914, IIHR-2627 and Arka Abha genotypes upon temperature stress as compared to other genotypes. Although, there was no significant difference in RWC during early stages of stress, it was significantly higher in IIHR-2841, IIHR-2202 and IIHR-2745 genotypes at ninth day. Epicuticular wax content increased significantly in genotypes IIHR-2202 and IIHR-2841 compared to other genotypes; whereas, lipid peroxidation increased significantly in genotypes Arka Abha and IIHR-2627. Based on these results, genotypes *viz.,* IIHR-2841 and IIHR-2202 were found to be temperature tolerant, while Arka Abha and IIHR-2914 were comparatively sensitive. The genotypes identified as tolerant to high temperature can act as a potential genetic material for future breeding programs to develop high yielding temperature tolerant varieties.

Keywords: Tomato genotypes, High temperature, Tolerant, Susceptible

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop widely grown all over the world. It is the number nine crop on the lists of food commodities and is the second most important vegetable crop in the world, next to potato and it is also widely used as a model crop for source-sink studies and stress. The growing food demand and the threat of heavy crop losses due to global climate change impose the need for urgent development of strategies to substantially improve food production. Enhancing crop productivity plays thus an important role in achieving breeder's goals. Agricultural production and productivity are predicted to be affected by increasing temperatures resulting from global warming (Ainsworth and Ort, 2010), and the most important goal of plant breeders is to develop high yielding varieties that are resistant to biotic and abiotic stress factors.

Elevated temperatures have become an increasingly serious agricultural problem in many regions of the world. Heat stress caused by elevated temperatures induces morphological, anatomical, physiological, biochemical and genetic responses in plants (Min *et al*., 2014), which further decrease crop yield and quality. However, the response and susceptibility of plants to high temperature vary between genotypes and developmental stages (Wahid *et al*., 2007). Variation in the response of genotypes to heat stress is not only observed in the vegetative organs (leaves) but also in the reproductive organs.

Based on preliminary studies, an efficient screening technique referred to as the temperature induction response (TIR) technique has been developed to identify thermotolerant lines. According to this technique, the seedlings are exposed to an optimum induction temperature before being exposed to a severe challenging temperature and subsequently allowed to recover at room temperature. The surviving seedlings at the end of the recovery period are selected as thermotolerant lines. Earlier studies clearly showed that TIR is an effective technique for screening for high temperature tolerance.

Heat tolerance in plants is growth stage specific. Differential temperature sensitivity for vegetative and reproductive growth has been reported in various species. In tomato, the optimal temperature for growth is between 25 and 30°C, whereas, the optimal temperature for fruit set is 22-25°C. Cultivation of tomatoes under higher temperatures than the optimum has a negative impact on plant growth, decreasing productivity and ultimately yield. As a consequence of global warming, the impact of high temperatures on field-grown tomatoes has become a critical issue to resolve. A thorough understanding of mechanisms of temperature tolerance and physiological responses in tomatoes is thus imperative for maintenance and development of future crop systems.

The reproductive phase is regarded as the most sensitive stage to heat stress in tomato. The floral organs were most adversely affected at the initial stages of development (Wahid *et al*., 2007). Heat tolerance at the anthesis stage is important as anthesis is the key stage for the determination of the final yield. However, there is limited knowledge on the effects of heat stress during the early and later stages of plant growth. In this paper, the physiological responses of tomato genotypes with different responses identified through TIR studies were evaluated at flowering / fruiting stage. This study was performed to identify the physiological responses of tomato genotypes to high temperature stress in terms of membrane stability index, lipid peroxidation, wax content, relative water content and specific leaf area at flowering stage. Such an information help us to better understand the mechanisms of the physiological responses of tomato genotypes to high temperature stress and establish the relation between the temperature stress responses of tomato at the vegetative and reproductive growth stages.

Material and Methods

Plant material, growth condition and high temperature treatment

On the basis of temperature induction response (TIR), three tolerant genotypes *viz.,* IIHR-2202, IIHR-2745 and IIHR-2841 and three susceptible genotypes *viz.,* Arka Abha, IIHR-2627 and IIHR-2914 were used for evaluating their performance under high temperature stress condition. The experiment was carried out at ICAR-Indian Institute of Horticultural Research, Bengaluru during the months of February to June (summer) 2017. Bengaluru is located at 13°58' N latitude, 78°E longitude and 890 m above mean sea level. Seeds were sown in protrays during the first week of February, 2017 and seedlings were transplanted in the field after 30 days of sowing. The experiment was laid out in completely randomized block design with five replications. Recommended agronomic practices and plant protection measures were followed to raise the crop. At the early flowering stage (30 DAT), the temperature stress (40±2°C) was imposed using polytunnel (Plate 1). Various physiological and biochemical parameters were assessed in tomato plants grown under both control and temperature stress treatments at second, fifth and nine days after imposition of stress.

Plate 1 : Experimental layout of tomato genotypes in the field (a) Control and (b) High temperature stress

Leaf membrane stability index (MSI)

MSI was determined according to the method of Premachandra *et al*. (1991). MSI was estimated by taking ten leaf discs in 25 ml. of double-distilled water and was heated at 50°C for 30 min in a water bath and measured for electrical conductivity (C1). Then it was boiled at 100°C for 10 min prior to having its conductivity (C2) measured. MSI was calculated using the formula MSI = [1- (C1 / C2)] x 100 where,

C1 and C2 are the electric conductivities recorded at 50 °C and 100 °C, respectively.

Lipid peroxidation

Lipid peroxidation was estimated by determining the concentration of malondialdehyde (MDA) produced by the thiobarbituric acid (TBA) reaction. This experiment was performed following the method described by Hodges *et al*. (1999). Leaf material (1.0 g) was homogenized in 5 ml of 5 per cent aqueous TCA and 0.5 ml of 0.5 per cent methanolic BHT and heated for 30 minutes in a boiling water then the sample was centrifuged at 10000 rpm for 10 minutes Then to 1 ml of supernatant sample and 1 ml saturated TBA solution was added and kept in a boiling water bath for 30 min. Centrifuged at 10000 rpm for 10 min. and the absorbance was read at 532 nm. The MDA (mg/100g) concentration was derived according to the following equations :

$$MDA = \frac{OD_{532nm} \times \text{Std. value } (\mu g) \times \text{Total Vol. of extract} \times 100}{\text{Assay volume} \times \text{Wt. of the sample (g)} \times 1000}$$

Relative water content (RWC)

RWC is a useful indicator of the state of water balance of a plant essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation. Leaves were immediately weighed (fresh weight) and then were floated in distilled water inside a closed petri dish for four hours. The petri dishes were maintained under dim light, after gently wiping the water from the leaf surface with tissue paper, took the weight in order to obtain the turgid weight. These leaves were placed in oven at 80 ^{0}C for 48 hours in order to get the dry weight. RWC was calculated by using the formula, RWC (%) = [(Fresh weight – Dry weight) / (Turgid weight - Dry weight)]* 100.

Epicuticular wax

Epicuticular wax was estimated by using the method of Ebercon *et al.* (1977). Leaf samples of uniform area (2cm^2) immersed in 15 ml of chloroform for 15 sec at room temperature. The extract was evaporated to dryness in a water bath at 70 °C. After adding 5 ml acidic $K_2Cr_2O_7$, the solution was heated in a boiling water bath for 30 min. After cooling, 5 ml distilled water was added and the optical density read at 590 nm. Concentration of leaf wax was calculated from a standard curve prepared using Nonadecane.

Specific leaf area (SLA)

The determination of leaf area is essential to understand the interaction between plant development and prevailing environmental factors during the growing season (Dannehl *et al*., 2015). Leaves with known area were kept in hot air oven for drying for 48 hours. Dry weight was taken and calculated the SLA using the formula: Leaf area / Dry weight.

Statistical analysis

Results were analyzed using analysis of variance and standard error was calculated accordingly.

Results and Discussion

The effect of high temperature stress on membrane stability index and specific leaf area in contrasting tomato genotypes are given in Fig. 1 and 2, respectively. MSI decreased significantly in IIHR-2914, IIHR-2627 and Arka Abha genotypes upon heat stress as compared to other three genotypes. The rate of injury to cell membranes by heat was estimated through measurement of electrolyte leakage from the cells. The MSI is commonly used as a measure of tolerance to plant stresses such as freezing and heat. The varieties showing high membrane thermostability

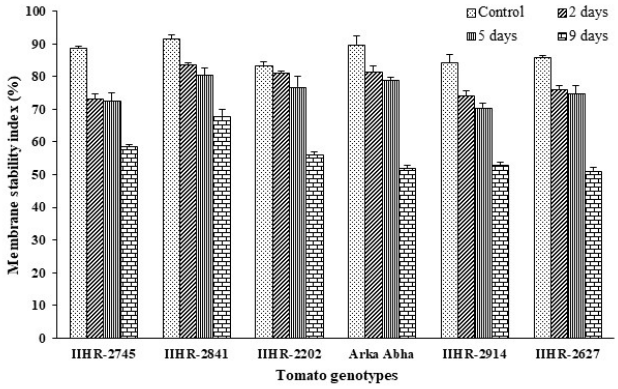

Fig. 1 : Membrane stability Index (MSI) under control and temperature stress conditions in tomato genotypes

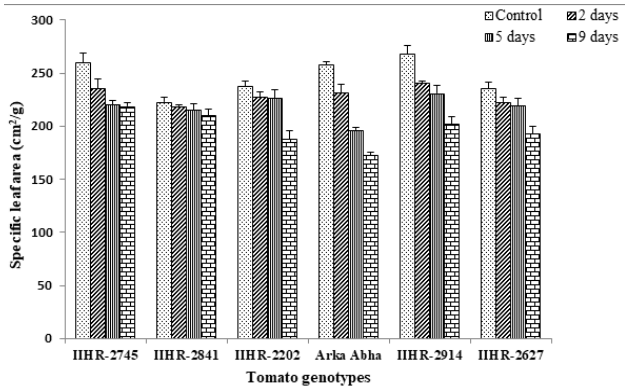

Fig. 2 : Specific leaf area (SLA) under control and temperature stress conditions in tomato genotypes

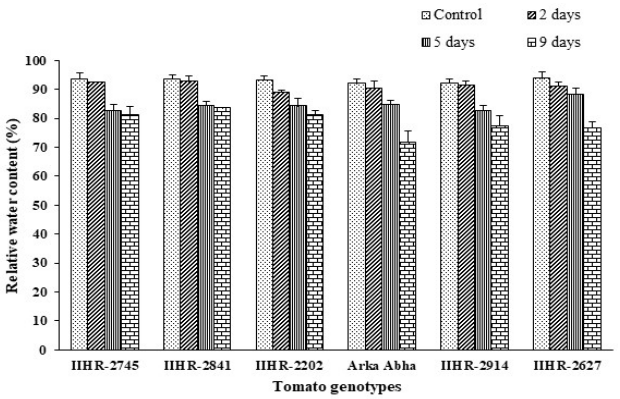

Fig. 3 : Relative water content (RWC) under control and temperature stress conditions in tomato genotypes

values are more likely to tolerate high temperature and relatively longer exposure to high temperature would cause damage to leaf tissues (Saeed *et al*., 2007).

High specific leaf area reflects a fundamental trait for a rapid production of biomass. It has thus been proposed that this trait be measured routinely in screening programmes. In the earlier study it is observed that SLA was decreased in droughted amaranth plants compared to well-watered plants. The extent of reduction in SLA indicates the effect of temperature stress on leaf expansion rate. Higher reduction in the susceptible genotypes under stress condition indicated significant reduction in leaf expansion rate at different days of temperature stress and such a reduction was not observed in tolerant genotypes. In IIHR-2841 genotype, SLA was not significantly differ between control and the different days of temperature stress, there by SLA also plays a major role in heat tolerance.

The RWC is a useful indicator of the state of water balance of a plant and it is essential because it expresses the absolute amount of water which the plant requires to reach full saturation. Although, there was no significant difference in RWC during early stages of stress but at ninth day after imposition of stress, the genotypes IIHR-2841, IIHR-2202 and IIHR-2745 had significantly higher RWC compared to susceptible genotypes (Fig. 3) and this was also evidenced by the data in wheat cultivars (Sarkar *et al*., 2016).

The ability of a plant to survive under high temperature conditions depends in part on its ability to reduce the amount of irradiation entering the leaf and water loss through both stomatal and epidermal transpiration. Epicuticular wax (EW) is an important adaptive trait that covers aerial surfaces forming a barrier between the environment and the plant, offering protection against both abiotic stresses, such as excess irradiation, water loss, high temperatures and biotic stresses (Shepherd and Griffiths, 2006). Previously, it has been observed that high temperature stress increased the amount of EW deposited on the leaf surface in wheat and carnation. High temperature also influences the composition of EW by altering its hydrocarbons content. EW can reduce the amount of irradiation entering the leaf, thereby reducing thermal load by reflecting excess light and by providing a barrier against water loss through epidermal transpiration in hot and dry environments. (Kim *et al.*, 2007). In the study, EW content increased significantly in the genotypes IIHR-2202 and IIHR-2841 which are highly tolerant to high temperature compared to the susceptible genotypes IIHR-2914 and Arka Abha (Table 1).

Heat stress results in production of excessive reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen. ROS produced within plant tissue by heat stress damages membrane by lipid peroxidation forming minute hydrocarbon fragments, like malondialdehyde (MDA), one of the important indicators of oxidative

TABLE 1

Epicuticular wax content (mg/cm^2) under control and temperature stress conditions in tomato genotypes

Genotypes/Treatment	Control	2nd day Stress	5th day Stress	9th day Stress
IIHR-2745	0.046 ± 0.004	0.055 ± 0.003	0.065 ± 0.003	0.068 ± 0.006
IIHR-2841	0.047 ± 0.005	0.065 ± 0.002	0.065 ± 0.006	0.068 ± 0.006
IIHR-2202	0.036 ± 0.003	0.045 ± 0.006	0.066 ± 0.004	0.067 ± 0.007
Arka Abha	0.033 ± 0.002	0.036 ± 0.001	0.050 ± 0.003	0.060 ± 0.002
IIHR-2914	0.041 ± 0.003	0.047 ± 0.005	0.050 ± 0.000	0.051 ± 0.002
IIHR-2627	0.043 ± 0.002	0.052 ± 0.005	0.056 ± 0.003	0.060 ± 0.003
Mean	0.041 ± 0.003	0.050 ± 0.004	0.059 ± 0.003	0.062 ± 0.004
CD for G ($p \leq 0.05$)			0.006	
CD for T ($p \leq 0.05$)			0.005	
CD for GxT ($p \leq 0.05$)			0.011	

TABLE 2

Malondialdehyde content (mg/100g fresh weight) under control and temperature stress conditions in tomato genotypes

Genotypes/Treatment	Control	2nd day Stress	5th day Stress	9th day Stress
IIHR-2745	0.183 ± 0.001	0.245 ± 0.012	0.460 ± 0.012	0.572 ± 0.010
IIHR-2841	0.114 ± 0.014	0.134 ± 0.018	0.285 ± 0.018	0.447 ± 0.013
IIHR-2202	0.168 ± 0.006	0.314 ± 0.043	0.444 ± 0.006	0.557 ± 0.019
Arka Abha	0.119 ± 0.007	0.175 ± 0.010	0.428 ± 0.015	0.625 ± 0.004
IIHR-2914	0.286 ± 0.007	0.396 ± 0.018	0.460 ± 0.039	0.583 ± 0.009
IIHR-2627	0.120 ± 0.021	0.177 ± 0.012	0.385 ± 0.005	0.599 ± 0.017
Mean	0.165 ± 0.009	0.240 ± 0.019	0.410 ± 0.016	0.564 ± 0.012
CD for G ($p \leq 0.05$)		0.024		
CD for T ($p \leq 0.05$)		0.020		
CD for GxT ($p \leq 0.05$)		0.048		

stress. Sarkar *et al.* (2016) observed that temperature stress enhanced membrane peroxidation as reflected by the elevated level of MDA in contrasting wheat cultivars. In the present experiment, MDA content increased significantly in temperature susceptible genotypes Arka Abha and IIHR-2627 as compared to the tolerant genotypes (Table 2).

The study clearly indicated that out of the six genotypes, IIHR-2841 and IIHR-2202 showed a much more distinct antioxidative tolerance mechanism after the imposition of heat stress, and therefore, they seemed to be protected from the harmful damage caused by oxidative stress as a result of heat stress and increased severity of stress. It was also found that these tolerant genotypes were able to withstand the heat stress more effectively than the other three susceptible genotypes. Out of the susceptible genotypes, Arka Abha was more sensitive to heat stress than the other genotypes. The study helped us to further characterize the tomato genotypes based on physiological parameters and identify the basis of tolerance.

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