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M Arumugaperumal
MSc graduate, Tamil Nadu Agricultural University Coimbatore, Tamil Nadu, India

KB Sujatha
Assistant Professor (Crop Physiology), Department of Fruit Crops, Horticultural College & Research Institute Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
M Arumugaperumal
MSc graduate, Tamil Nadu Agricultural University Coimbatore, Tamil Nadu, India

Response of antioxidant enzymes in tomato genotypes exposed to elevated temperature

M Arumugaperumal and KB Sujatha

Abstract
Tomato (Solanum lycopersicon) is one of the most important and widely grown vegetables crops in both temperate and tropical regions of the world. Global climate models projects an increase in temperature of 0.6–4.8ºC that is anticipated to be one of the limiting factors for cultivation of tomato and other plants in the future. In this study, plants were grown under two different temperature regimes, one at ambient (30 °C) and other at elevated temperature (38±1ºC) in open top chambers at the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. The antioxidant enzyme activity of catalase (CAT), peroxidase (POX) and super oxide dismutase (SOD) of 20 tomato genotypes to elevated temperature were evaluated. The present study revealed that the genotype IIVR – L showed a higher activity of catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) and their activity was lowest in IIHR-709 and IIHR-2388. The genotype IIVR-L with higher accumulation of antioxidant enzymes was able to detoxify reactive oxygen species and mitigate oxidative stress-induced damage under elevated temperature whereas it was low in IIHR-709 and IIHR-2388 which was evident from the better yield in IIVR-L compared to the poorly performed genotypes (IIHR-2388 and IIHR–709).

Keywords: Tomato, elevated temperature, Antioxidant enzymes, Yield

Introduction
Tomato (*Solanum lycopersicon* L.) is one of the most significant vegetable and it is typically cultivated for its edible fruit. India is the second largest producer of tomato after China in the world after potato and sweet potato both in area and production (NHB, 2014). The global average surface temperature is predicted to increase by 0.6–4.8ºC compared with the beginning of this century (IPCC, 2007) In addition to mean increase in annual temperatures, there will also be increase in the frequency, duration, and severity of periods with exceptionally high temperatures (i.e., heat waves) (Haldimann and Feller, 2004) [9]. Thus, in future, plants are likely to undergo an increases in heat stress which can impact plant growth and development, decreasing crop and ecosystem productivity and biodiversity (Thomas *et al*., 2004) [18].

High temperature stress induces the rapid production and accumulation of reactive oxygen species (ROS) (Mittler, 2002; Almeselmani *et al*., 2009). Reactive oxygen species (ROS) are produced continuously as byproducts of different metabolic pathways which are located in different cellular compartments such as chloroplast, mitochondria and peroxisomes. Through a variety of reactions, O_2^- leads to the formation of H_2O_2, OH^- and other ROS. The ROS comprising O_2^-, H_2O_2, $^1O_2$, HO_2^-, OH^-, ROOH, ROO^+ and RO^+ are highly reactive and toxic and causes damage to proteins, lipids, carbohydrates and DNA which ultimately results in cell death. Accumulation of ROS as a result of high temperature stress is a major cause of loss of crop productivity worldwide. (Mittler, 2002; Apel and Hirt, 2004) [3].

The detoxification of these ROS is very important and plants have evolved complex strategies to deal with them (Asthir *et al*., 2012) [4]. The plant cells typically respond to increases in ROS levels by increasing the expression and activity of ROS scavenging enzymes and increasing their production of antioxidants in order to maintain redox homeostasis. The major enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) are reported to increase under various environmental stress (Munne-Bosch and Alegre, 2000). In enzymatic antioxidant system, SOD converts free $O2^-$ radicals to H_2O_2 and O_2. The catalase and ascorbate peroxidase scavenge the accumulated H_2O_2 to nontoxic levels or form water and oxygen (Mittler, 2002).

There are various reports on the effect of high temperature on antioxidant enzymes. Heat shock pre-treatment induced significant increase in SOD activity when compared to negative control in greengram seedlings. In another study, reduction in SOD activity due to high temperature (45°C) was reported in cotton (*Gossypiumhirsutum* L.) by Gur *et al*. (2010). On

the other hand, increase in SOD activity as a result of heat stress was reported by Jiang and Huang (2001) [10]; such increase could be due to the accumulation of Cu/Zn-SOD mRNA (Reddy *et al*., 2004) reported that comparatively higher activity has been observed in tolerant cultivars than in sensitive ones, indicating that higher antioxidant enzymes activity have a role in imparting tolerance to these cultivars against environmental stress.

Catalase (CAT) scavenges H_2O_2 to nontoxic levels or catalyze the formation of water and oxygen, an increase in CAT activity could play a role in the protection of the plants from the damages of upward accumulation of H_2O_2. A significant increase in CAT activity (45.86%) in the cotton plants treated with 45°C temperature and increased CAT activities in *T. aestivum* genotypes to heat treatment (Keles and Oncel, 2002) [11]. Similar results were observed in Kentucky bluegrass where CAT activity reached up to the maximum level after a ½ and 1 h of heat treatments and thereafter decreased. One of the major reasons for increase in CAT activity was the excess generation of reactive oxygen species (ROS) that leads to oxidative stress. Peroxidases are involved in many physiological processes in plants, involving responses to biotic and abiotic stresses and the biosynthesis of lignin. They are also involved in the scavenging of ROS, which are partially reduced forms of atmospheric oxygen, highly reactive, and capable of causing oxidative damage to the cell. Peroxidases can be a source of hydrogen peroxide (H_2O_2) but also are capable of scavenging it. POX enzyme has been related to the appearance of physiological injuries caused in plants by thermal stress, and its activity was enhanced by high temperature stress (Mazorra *et al*., 2002) [12]. Hence, understanding the response of tomato to high temperature at antioxidant enzyme level will accumulate knowledge, making the genetic manipulation easier which could lead to more research on mechanism of high temperature stress tolerance to plants in future.

Materials and Methods

The present study was carried out to study the genetic variation in 20 tomato genotypes in their ability to scavenge the reactive oxygen species produced under elevated temperature. The pot culture experiment with 20 genotypes were carried out at the Department of Crop Physiology (11º N latitude, 77º E longitude; 426.7 MSL), TamilNadu Agricultural University, Coimbatore to and the experimental period was 27th November 2015 to 4th April 2016. Recommended dose of fertilizers and common package of practices were followed and each genotype was replicated thrice in a completely randomized block design. The seeds were treated with Carbendazim @ 0.5g kg^{-1} of seeds for protection against seed borne diseases. The seeds were sown uniformly in the well prepared portrays maintaining a thin film of water. Twenty days after sowing, uniform seedlings were transplanted to pots with recommended soil proportion. Two sets of pots one for elevated temperature and the other for ambient temperature were maintained for each genotype. The pots were shifted to ambient and elevated temperature chambers after 10 DAT (days after transplanting). Recommended management and plant protection measures were followed.

The two open top temperature controlled chambers with the dimensions of 3 m x 3 m were fabricated for this study. This chamber was made using poly carbonate sheets recommended for crop growth experiments. The air temperature of the chamber was maintained automatically with controller using PT100 thermostat sensor. The generation of heat was manipulated by heater fixed outside the chamber. Once the required temperature (38ºC) is reached, the controller will automatically shut off the heater and blower. The standard deviation of temperature was +/-0.5 ºC. One chamber was maintained at an ambient temperature of 30ºC ± 1ºC (T1) and the other chamber was maintained at an elevated temperature of 38 ± 1ºC (T2) for a duration of 6 hours from 10 am to 4 pm. Twenty tomato genotypes were sown in portrays and transplanted in pots after twenty days of sowing. They were transferred to the open top chambers after 10 DAT and irrigation was given at 50 percent available soil moisture (ASM).

The observations were recorded at different plant growth stages viz., 30 DAT, 60 DAT and 90 DAT by selecting samples randomly from each replication. Peroxidase (POX) (change in OD value at 430 nm g^{-1} $min^{-1)}$ was determined according to Perur (1962) [15] and Angelini *et al.* (1990) [2]. Catalase (CAT) activity was assayed from the rate of H_2O_2 decomposition extinction coefficient of 39.4 mmol as measured by the decrease in the absorbance at 240 nm, following the procedure of Aebi (1974). Superoxide Dismutase (SOD) activity was determined by using nitro blue tetrazolium (NBT) salt as described by Champ and Fridovich (1971) [7] and expressed in enzyme units mg g^{-1} of protein. The total number of fruits in each plant was counted to get fruit number per plant or the plant yield.

Results and Discussion

Plants use antioxidant enzymes to detoxify reactive oxygen species and mitigate oxidative stress-induced damage under elevated temperature (Shah *et al*., 2001) [17]. Activities of different antioxidant enzymes are temperature sensitive and activation occurs at different temperature ranges but the activities of these enzymes increase with increasing temperature (Chakraborty and Pradhan, 2011) [6]. Many studies demonstrated that high temperature injury was caused by the excessive production of reactive oxygen radicals, the low activities of antioxidant enzymes, and the membrane damage in plants (Zhang *et al*., 2006). Decrease in antioxidant activity in stressed tissues results in higher levels of ROS that may contribute to injury (Fadzillah *et al*., 1996) [8]. The present study reveals that significant genotypic differences were observed in the catalase activity of the leaves during flowering stage under ambient and elevated temperature (Table 1). Higher catalase activity was recorded in IIVR - L with a value of 11.25, 8.58 µg of and 7.08 µg of H_2O_2 g^{-1} min^{-1} under ambient temperature and 10.0, 6.80 and 4.38 µg of H_2O_2 g^{-1} min^{-1} of under elevated temperature at 30 DAT,60 DAT and 90 DAT. Interaction effect was significant at all the stages of observation.

A possible mechanism that may be involved in the resistance to many types of stress is the increased activity of the antioxidant enzymes. Higher activity of antioxidant enzymes has been found in response to abiotic stress, and they may have a general role in the acquisition of tolerance of plants to different environmental stress. The present experiment showed that peroxidase activity was significantly increased under elevated temperature compared to ambient condition (Table 2). The genotypes IIVR-L recorded the highest peroxidase activity of 1.96, 2.46 and 2.16 absorption at 430 nm min^{-1} g^{-1} under ambient condition and it was 2.40, 3.15 and 2.55 absorption at 430 nm min^{-1} g^{-1} under elevated

temperature condition at 30 DAT, 60 DAT and 90 DAT respectively. At ambient temperature condition, peroxidase activity of IIVR – L was on par with EC – 608395 at 30 DAT and 90 DAT. Genotype and temperature interaction was significant at all the stages of plant growth.

Superoxide dismutase activity increased trend under elevated temperature compared to ambient condition (Table 3). The genotype IIVR – L however has showed a higher degree of accumulation of SOD. The enzyme SOD activity was significantly high in IIVR – L whereas it was very low in IIHR-709 and IIHR-2388. The genotype IIVR – L recorded 0.88, 1.03 and 1.16 unit mg^{-1} of protein of SOD activity under ambient temperature and 1.01, 1.34 and 1.79 unit mg^{-1} of protein under elevated temperature at 30 DAT, 60 DAT and 90 DAT respectively. Interaction of the genotype with temperature was not significant at 60 DAT

In all the genotypes, fruit yield per plant (g /plant) was significantly reduced by high temperature stress treatment among the genotypes, but degree of reduction was different among genotypes (Table 4). In IIVR – L (37.55 %) and LE – 1 (54.32%) yield reduction was small, while in some genotypes like EC – 177824 (79.68 %) LE – 114 (73.57 %) higher reduction was observed. Genotypes IIHR – 709 and IIHR – 2388 could not produce any yield under elevated temperature conditions. Fruit yield was highest in the genotypes IIVR – L followed by the genotypes LE – 3, LE – 1 in the control plants. In the stressed plants, the highest yield was found in the genotypes IIVR – L (295.87 %) followed by LE – 1, LE – 3 and LE – 114.

This study corroborates the earlier findings where the genotype IIVR – L recorded a higher activity of catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) and their activity was lowest in IIHR-709 and IIHR-2388 and manifests that high temperature stress induces the rapid production and accumulation of reactive oxygen species (Table 1,2&3). Reactive oxygen species (ROS) are produced continuously as byproducts of different metabolic pathways which are located in different cellular compartments such as chloroplast, mitochondria and peroxisomes (Cao *et al*., 2009) [5] resulting in a major yield loss (Mittler, 2002; Apel and Hirt, 2004) [3]. The detoxification of these ROS (Asthir *et al*., 2012) [4] is done by these antioxidant enzymes in tolerant species by scavenging the ROS (Munne -Bosch and Alegre, 2000). Thus the genotype IIVR-L with higher accumulation of antioxidant enzymes was able to detoxify reactive oxygen species and mitigate oxidative stress-induced damage under elevated temperature (Shah *et al*., 2001) [17] whereas IIHR-709 and IIHR-2388 with low antioxidant enzyme activity at elevated temperature suppressed the plants efficiency to tolerate the heat stress.

Table 1: Effect of elevated temperature on catalase activity (µg of H_2O_2 g^{-1} min^{-1}) in tomato genotypes under different stages of plant growth

S. No.	Genotypes	30 DAT			60 DAT			90 DAT		
		AT	ET	Mean	AT	ET	Mean	AT	ET	Mean
1.	LE – 114	12.83	11.92	12.38	10.16	8.72	9.44	8.66	6.30	7.48
2.	EC – 608456	12.50	11.44	11.97	9.83	8.24	9.04	8.33	5.82	7.08
3.	EC – 170047	12.41	11.25	11.83	9.74	8.05	8.90	8.24	5.63	6.94
4.	EC – 170089	12.70	11.54	12.12	10.03	8.34	9.19	8.53	5.92	7.23
5.	EC – 168290	12.66	11.52	12.09	9.99	8.32	9.16	8.49	5.90	7.20
6.	LE – 118	11.90	10.45	11.18	9.23	7.25	8.24	7.73	4.83	6.28
7.	LE – 1	12.05	11.22	11.64	9.38	8.02	8.70	7.88	5.60	6.74
8.	LE – 3	12.19	11.24	11.72	9.52	8.04	8.78	8.02	5.62	6.82
9.	IIHR – 709	13.00	12.19	12.60	10.33	8.99	9.66	8.83	6.57	7.70
10.	EC – 177360	12.69	11.53	12.11	10.02	8.33	9.18	8.52	5.91	7.22
11.	EC – 608395	11.50	10.22	10.86	8.83	7.02	7.93	7.33	4.60	5.97
12.	EC – 169966	11.54	10.40	10.97	8.87	7.20	8.04	7.37	4.78	6.08
13.	IIHR – 2388	13.20	12.88	13.04	10.53	9.68	10.11	9.03	7.26	8.15
14.	EC – 175957	12.79	11.80	12.30	10.12	8.60	9.36	8.62	6.18	7.40
15.	EC – 177325	12.60	11.50	12.05	9.93	8.30	9.12	8.43	5.88	7.16
16.	EC – 168283	12.74	11.67	12.21	10.07	8.47	9.27	8.57	6.05	7.31
17.	IIVR – L	11.25	10.00	10.63	8.58	6.80	7.69	7.08	4.38	5.73
18.	EC – 177824	12.90	11.99	12.45	10.23	8.79	9.51	8.73	6.37	7.55
19.	EC – 177371	12.88	11.93	12.41	10.21	8.73	9.47	8.71	6.31	7.51
20.	LE – 20	12.00	11.21	11.61	9.33	8.01	8.67	7.83	5.59	6.71
Mean		12.42	11.40	11.91	9.75	8.20	8.97	8.25	5.78	7.01
		G	**T**	**GxT**	**G**	**T**	**GxT**	**G**	**T**	**GxT**
SEd		0.19	0.06	0.27	0.13	0.04	0.18	0.08	0.02	0.12
CD (P=0.05)		0.39	0.12	0.55^{NS}	0.26	0.08	0.37^{NS}	0.17	0.05	0.24

AT: Ambient Temperature
ET: Elevated Temperature (38±1ºC)
G: Genotype
GxT: Interaction of genotype and Temperature

Table 2: Effect of elevated temperature on peroxidase (changes in absorption at 430 nm min^{-1} g^{-1}) in tomato genotypes under different stages of plant growth

S. No.	Genotypes	30 DAT			60 DAT			90 DAT		
		AT	ET	Mean	AT	ET	Mean	AT	ET	Mean
1.	LE – 114	1.56	1.62	1.59	2.06	2.37	2.22	1.76	1.77	1.77
2.	EC – 608456	1.66	1.74	1.70	2.16	2.49	2.33	1.86	1.89	1.88
3.	EC – 170047	1.66	1.76	1.71	2.16	2.51	2.34	1.86	1.91	1.89
4.	EC – 170089	1.53	1.60	1.57	2.03	2.35	2.19	1.73	1.75	1.74
5.	EC – 168290	1.60	1.77	1.69	2.10	2.52	2.31	1.80	1.92	1.86
6.	LE – 118	1.82	1.88	1.85	2.32	2.63	2.48	2.02	2.03	2.03
7.	LE – 1	1.74	1.78	1.76	2.24	2.53	2.39	1.94	1.93	1.94
8.	LE – 3	1.66	1.76	1.71	2.16	2.51	2.34	1.86	1.91	1.89
9.	IIHR – 709	1.48	1.56	1.52	1.98	2.31	2.15	1.68	1.71	1.70
10.	EC – 177360	1.47	1.55	1.51	1.97	2.30	2.14	1.67	1.70	1.69
11.	EC – 608395	1.96	2.24	2.10	2.46	2.99	2.73	2.16	2.39	2.28
12.	EC – 169966	1.82	1.89	1.86	2.32	2.64	2.48	2.02	2.04	2.03
13.	IIHR – 2388	1.25	1.33	1.29	1.75	2.08	1.92	1.45	1.48	1.47
14.	EC – 175957	1.38	1.45	1.42	1.88	2.20	2.04	1.58	1.60	1.59
15.	EC – 177325	1.56	1.62	1.59	2.06	2.37	2.22	1.76	1.77	1.77
16.	EC – 168283	1.40	1.49	1.45	1.90	2.24	2.07	1.60	1.64	1.62
17.	IIVR – L	1.96	2.40	2.18	2.46	3.15	2.81	2.16	2.55	2.36
18.	EC – 177824	1.30	1.39	1.34	1.80	2.14	1.97	1.50	1.54	1.52
19.	EC – 177371	1.33	1.40	1.37	1.83	2.15	1.99	1.53	1.55	1.54
20.	LE – 20	1.64	1.88	1.76	2.14	2.63	2.39	1.84	2.03	1.94
MEAN		8.02	1.71	4.86	2.09	2.46	2.27	1.79	1.86	1.82
		G	**T**	**GxT**	**G**	**T**	**GxT**	**G**	**T**	**GxT**
SEd		0.02	0.01	0.03	0.03	0.01	0.04	0.03	0.01	0.04
CD (P=0.05)		0.05	0.01	0.07	0.06	0.02	0.09	0.06	0.01	0.08

AT: Ambient Temperature
ET: Elevated Temperature (38±1ºC)
G: Genotype
GxT: Interaction of genotype and Temperature

Table 3: Effect of elevated temperature on superoxide dismutase (unit mg^{-1} of protein) in tomato genotypes under different stages of plant growth

S. No.	Genotypes	30 DAT			60 DAT			90 DAT		
		AT	ET	Mean	AT	ET	Mean	AT	ET	Mean
1.	LE – 114	0.57	0.62	0.60	0.72	0.95	0.84	0.85	1.40	1.13
2.	EC – 608456	0.69	0.77	0.73	0.84	1.10	0.97	0.97	1.55	1.26
3.	EC – 170047	0.70	0.79	0.75	0.85	1.12	0.99	0.98	1.57	1.28
4.	EC – 170089	0.60	0.68	0.64	0.75	1.01	0.88	0.88	1.46	1.17
5.	EC – 168290	0.64	0.74	0.69	0.79	1.07	0.93	0.92	1.52	1.22
6.	LE – 118	0.79	0.90	0.85	0.94	1.23	1.0	1.07	1.68	1.38
7.	LE – 1	0.75	0.85	0.80	0.90	1.18	1.04	1.03	1.63	1.33
8.	LE – 3	0.73	0.81	0.77	0.88	1.14	1.01	1.01	1.59	1.30
9.	IIHR – 709	0.46	0.53	0.50	0.61	0.86	0.74	0.74	1.31	1.03
10.	EC – 177360	0.62	0.72	0.67	0.77	1.05	0.91	0.90	1.50	1.20
11.	EC – 608395	0.85	0.97	0.91	1.00	1.30	1.15	1.13	1.75	1.44
12.	EC – 169966	0.80	0.94	0.87	0.95	1.27	1.11	1.08	1.72	1.40
13.	IIHR – 2388	0.45	0.51	0.48	0.60	0.84	0.72	0.73	1.29	1.01
14.	EC – 175957	0.58	0.63	0.61	0.73	0.96	0.85	0.86	1.41	1.14
15.	EC – 177325	0.65	0.75	0.70	0.80	1.08	0.94	0.93	1.53	1.23
16.	EC – 168283	0.59	0.65	0.62	0.74	0.98	0.86	0.87	1.43	1.15
17.	IIVR – L	0.88	1.01	0.95	1.03	1.34	1.19	1.16	1.79	1.48
18.	EC – 177824	0.53	0.59	0.56	0.68	0.92	0.80	0.81	1.37	1.09
19.	EC – 177371	0.55	0.60	0.58	0.70	0.93	0.82	0.83	1.38	1.11
20.	LE – 20	0.75	0.88	0.82	0.90	1.21	1.06	1.03	1.66	1.35
MEAN		0.66	0.75	0.70	0.81	1.08	0.94	0.94	1.53	1.23
		G	**T**	**GxT**	**G**	**T**	**GxT**	**G**	**T**	**GxT**
SEd		0.01	0.003	0.01	0.01	0.004	0.02	0.01	0.005	0.02
CD (P=0.05)		0.02	0.006	0.02	0.02	0.01	0.04^{NS}	0.03	0.01	0.05^{NS}

AT: Ambient Temperature
ET: Elevated Temperature (38±1ºC)
G: Genotype
GxT: Interaction of genotype and Temperature

Table 4: Effect of elevated temperature on yield per plant (g/plant) in different tomato genotypes

S. No.	Genotypes	Yield per plant (g/plant)		Mean
		AT	ET	
1.	LE – 114	388.23	223.32	305.78
2.	EC – 608456	409.45	238.23	323.84
3.	EC – 170047	384.95	230.63	307.79
4.	EC – 170089	389.09	228.74	308.92
5.	EC – 168290	381.37	227.39	304.38
6.	LE – 118	378.97	240.41	309.69
7.	LE – 1	394.82	243.31	319.07
8.	LE – 3	389.09	239.04	314.07
9.	IIHR – 709	378.34	0.00	189.17
10.	EC – 177360	367.35	222.38	294.87
11.	EC – 608395	369.68	241.70	305.69
12.	EC – 169966	340.74	221.72	281.23
13.	IIHR – 2388	331.81	0.00	165.91
14.	EC – 175957	438.42	241.78	340.10
15.	EC – 177325	330.21	210.32	270.27
16.	EC – 168283	352.73	216.84	284.79
17.	IIVR – L	453.52	295.87	374.70
18.	EC – 177824	394.82	219.27	307.05
19.	EC – 177371	402.24	224.31	313.28
20.	LE – 20	363.50	232.12	297.81
MEAN		381.97	209.87	295.92
		G	**T**	**GxT**
SEd		4.49	1.42	6.36
CD (P=0.05)		8.95	2.83	12.66

AT: Ambient Temperature
ET: Elevated Temperature (38±1ºC)
G: Genotype
GxT: Interaction of genotype and Temperature

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