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SHORT COMMUNICATION

Antioxidant enzymes activity and physiological response of tomato (*Lycopersicon esculentum* M.) genotypes under mild temperature stress

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Abstract Tomato being sensitive to high temperature experiences mild to high temperature stresses under climate change conditions. To understand the response of tomato genotypes to mild temperature stress, a study was conducted in temperature gradient tunnel facility. The results revealed that across the genotypes studied, specific activity of antioxidant enzymes viz., superoxide dismutase (SOD), peroxidase (POX) and glutathione reductase (GR) increased significantly. Among the genotypes, increase in SOD activity was highest in cv. Arka Vikas, followed by IIHR 2195 and least in Abhinava. The GR activity was highest in Abhinava, followed by IIHR 2195 and least in cv. Arka Vikas. The mild temperature stress caused reduction in catalase (CAT) activity. The decrease in CAT activity and concomitant increase in POX activity was observed in cv. Arka Vikas. Low leaf water potential (Ψ_{leaf}) and higher electrolyte leakage indicated that the membrane integrity was affected across the tomato genotypes even under mild temperature stress. Among the genotypes studied, cv. Arka Vikas showed greater activity of SOD and POX, higher membrane stability and least reduction in water potential under mild temperature stress.

Keywords Climate change · Tomato · Temperature gradient tunnel · Scavenging enzyme activity · Physiological traits

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Genetic yield potential of crops is seldom attained under field conditions, mainly due to occurrence of abiotic stresses at critical growth stages. Successful cultivation of crops and realizing their potential yields is further threatened by high temperature and water deficit episodes associated with climate change. The multi model averages indicate daily maximum temperature increase of about 1–3 °C by middle of 21st century and about 2–5 °C by late 21st century (IPCC 2012). Under climate change conditions, depending on the growing season and region, crops are likely to experience mild to high temperature stresses coinciding with critical growth stages. Tomato is sensitive to high temperature, as it requires optimal mean daily temperatures between 21 and 24 °C, depending on developmental stage (Geisenberg and Stewart 1986). Mild temperature stress has been reported to reduced pollen viability, number of pollen released, fruit production, seed set, photosynthesis rate, photochemical efficiency of PSII in tomato (Peet et al. 1997; Sato et al. 2006; Islam 2011). These studies have also shown that the tomato genotypes have differential response to high temperature stress.

During temperature stress plants experience oxidative stress induced by reactive oxygen species (ROS), and it is the major cause of injury (Almeselmani et al. 2006). The levels of ROS in plants are regulated by complex antioxidant systems, both enzyme and non-enzymatic agents. Antioxidant enzyme systems are very important as a

defence mechanism to protect cellular membranes and organelles against ROS generated by environmental stresses in plants (Parvaiz et al. 2008).

The studies on the effect of high temperature stress have shown alterations in activities of antioxidant enzymes. High temperature stress of 35 °C caused increase in SOD activity and decrease in activity of CAT, POX, APX, dehydroascorbate reductase and GR and increased levels of antioxidant compounds (Rivero et al. 2004). Rainwater et al. (1996) and Rivero et al. (2004) reported that mild temperature stress increases the activity of antioxidant enzymes to cope up with the initial temperature stress.

In India, tomato is an important horticultural crop with the distinction of being the second largest produced vegetable. Presently in India tomato production is 186.53 lakh tons from an area of 9.07 lakh ha (NHB 2012). Many of the tomato producing regions in India are experiencing change in temperatures and sustaining yield levels would be further challenged by high temperature episodes under climate change conditions. Information on effect of mild temperature stress on Indian tomato genotypes with an emphasis on antioxidant enzyme activity and physiological response is scarce. Hence, the present experiment was conducted to study activity of antioxidant enzymes and physiological response of tomato genotypes under mild temperature stress as part of characterizing tomato genotypes for high temperature stress tolerance.

An experiment was carried out during October 2011 to February 2012 in the temperature gradient tunnel (TGT) facility established at Indian Institute of Horticulture Research (IIHR), Bangalore, Karnataka, India (13.15°N and 77.49°E elevation ~890 m). The TGT has dimensions of 18 m length, 4.5 m width and 3 m height and is covered with polycarbonate sheet. Three semi determinate tomato (*Lycopersicon esculentum* Mill.) genotypes, viz., Abhinava, IIHR 2195 and cv. Arka Vikas were selected for the study. Cultivar Arka Vikas was selected as it is suitable for cultivation in both rainy (*Kharif*) and winter (*Rabi*) seasons (Yadav 1998), and tolerant to high temperature. Advanced breeding line IIHR 2195 was selected based on its better performance during summer season. For comparison, commercial hybrid Abhinava, which is suitable for summer season was included. The seedlings were raised in portrays with coco peat as the growing medium. Twenty-five days old seedlings were transplanted to 20 litre capacity plastic containers filled with soil, FYM and sand in the ratio of 2:1:1. One week after transplanting, the containers were shifted inside the TGT for imposition of temperature treatments. A set of three genotypes containing six plants each was placed near the cooling pad and another set was placed towards the fan where the average air temperature was about 2 °C higher. The temperatures in each region were maintained only during the day time as the TGT worked on pad and fan system and temperature increase was due to the incoming solar radiation. The recommended fertilizer dose was applied to the seedlings and crop protection measures were taken as and when required.

Samples from fully opened leaf were collected between 0900 and 1000 h at flowering stage (41 days after transplanting), when the plants were experiencing air temperatures of 32.5 and 30.4 °C in the mild temperature stress treatment and control, respectively. Though the air temperature inside the TGT was 32.5 °C, the average leaf temperature recorded while measuring gas exchange rates using Portable Photosynthesis System, LI-6400 Xt (LiCor. Lincoln, Nebraska, USA) was 34.6 °C. Leaf tissue (0.5 g) was ground to a fine powder with mortar and pestle using liquid nitrogen. Enzyme extraction was done in potassium phosphate buffer (50 mM; pH 7.0) containing EDTA (1 mM), ascorbate (2 mM) and soluble PVP (1 %). The homogenate was centrifuged (HERMLE Z300 K, Germany) at 12,000 RPM for 15 min at 4 °C and the supernatant was used for the assay of antioxidant enzymes, viz., SOD, CAT, POX and GR using UV/VIS spectrophotometer (T80+UV/VIS Spectrometer; PG Instruments, UK). The activity of SOD (EC 1.15.1.1) was determined according to the method of Zhanyuan and Bramlage (1994). One unit of SOD activity was defined as the amount of enzyme required to bring about a 50 % inhibition in the absorption of formazone formed by the reduction of nitro blue tetrazolium by superoxide radical at 560 nm. The POX (EC 1.11.1.7) activity was measured as the absorbance due to tetraguaiacol at 450 nm according to the method of Subhas (1990) and expressed in units per mg of protein using standard enzyme (Sigma-Aldrich). The CAT activity was determined by a decline in absorbance at 240 nm (Masia 1998) expressed in units per mg of protein using standard enzyme (Sigma-Aldrich) and GR activity was assayed in terms of decline in the absorbance at 340 nm (Mavis and Stellwagen 1968) and enzyme activity was expressed in units per mg of protein using 6.22 mM as extinction co-efficient of NADPH.

The extent of membrane damage was estimated through percent electrolyte leakage. Leaf discs of 10 mm diameter were collected and incubated in 20 ml distilled water for 3 hrs at 25 °C and initial electrical conductivity (EC*a*) was measured using conductivity meter (SYSTRONICS; India). After 30 min of incubation in hot water bath at 55 °C EC*b* was recorded. Further leaf tissue was incubated in hot water bath for another 30 min at 100 °C and final EC*c* was recorded. The percent electrolyte leakage was calculated using the following formula.

$$\text{Electrolyte leakage } (\%) = (\text{EC}b - \text{EC}a) \div (\text{EC}c - \text{EC}a) \times 100$$

Table 1 Activity of superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT) and peroxidase (POD), per cent electrolyte leakage and water potential (Ψ leaf) in tomato genotypes grown at two temperature regimes

	SOD (units mg^{-1} protein)		GR (units mg^{-1} protein)		CAT (units mg^{-1} protein)		POX (units mg^{-1} protein)		electrolyte leakage (%)		Ψ leaf (Mpa)	
	Temperature (°C)											
	30.4	32.5	30.4	32.5	30.4	32.5	30.4	32.5	30.4	32.5	30.4	32.5
Abhinava	3.8a	4.3b	0.006b	0.035a	3.2b	2.3a	0.0015b	0.0036c	65.4b	78.7a	−0.65b	−1.23a
IIHR 2195	3.5a	4.7b	0.007b	0.024b	2.1c	0.7b	0.0037a	0.0070a	70.7ab	78.1a	−0.75b	−1.30a
Arka Vikas	3.9a	5.8a	0.019a	0.023b	5.5a	0.4b	0.0011c	0.0052b	73.4a	78.1a	−0.97a	−1.12a
Mean	3.7	4.9	0.010	0.028	3.6	1.1	0.0021	0.053	69.8	78.3	−0.79	−1.22
CD ($P = 0.01$)												
Treatments (T)	0.45		0.003		0.45		0.003		3.91		0.13	
Genotype (G)	0.56		0.003		0.56		0.003		NS		NS	
TxG	0.79		0.005		0.79		0.005		NS		0.22	
SE (m)±	0.19		0.003		0.19		0.003		1.33		0.61	

Means followed by the same letter in a column do not differ significantly according to Duncan's multiple-range test

The water potential of the third fully expanded leaf was determined using pressure bomb apparatus (ARIMAD 3000 MRC; USA). Statistical analysis was performed and tabulated using AGRIS STAT software for critical difference (CD) values. The Duncan multiple-Range comparison test was performed using the SPSS computer package (SPSS Inc. version 16.0) for all set of data.

Exposure of tomato genotypes to mild temperature increase in the TGT caused increase in activities of SOD, GR and POX. Though the air temperature inside the TGT was 32.5 °C, the average leaf temperature was 34.6 °C and tomato plants were experiencing mild temperature stress. The higher leaf temperature relative to the air temperature inside the TGT could be due to heating of leaves by incoming solar radiations. Under this mild temperature stress, increase in SOD activity was highest in cv. Arka Vikas and least in Abhinava (Table 1). Highest increase in GR activity was observed in Abhinava and least in cv. Arka Vikas (Table 1). Previous studies have shown that in tomato mild temperature stress of 34 °C increased the activity of SOD, CAT, POX and GR in heat tolerant genotypes of tomato (Rainwater et al. 1996).

In the present study, the mild temperature stress caused reduction in CAT activity in all the genotypes tested (Table 1). However, greater decrease in CAT activity and concomitant increase in POX activity were observed in cv. Arka Vikas (Table 1). It has been reported that increase in CAT activity compensated for decrease in POX activity in C3 and C4 plants (Foyer 2002) and under high temperature stress inactivation of CAT has been reported in tomato (Rivero et al. 2004).

Free radical induced peroxidation of membrane lipid is a reflection of stress induced damage at the cellular level. Therefore, relative electrolyte leakage has been widely used as a criterion to assess tolerance to heat injury in various crops. In the present study, electrolyte leakage showed significant increase across genotypes under mild temperature stress, indicating cellular injury due to ROS. Highest electrolyte leakage was observed in Abhinava and least in cv. Arka Vikas (Table 1). The results are consistent with previous reports for tomato (Camejo et al. 2005).

Mild temperature stress caused reduction in leaf water potential among all the genotypes. Leaf water potential reduction was least in cv. Arka Vikas and highest in Abhinava and IIHR 2195 (Table 1). (Morales et al. (2003) reported substantial reduction in water potential during heat stress in tomato plants. The effective removal of accumulated ROS by enzymatic quenching activities helps to maintain the photosynthesis and growth in tomato genotypes (Camejo et al. 2002; Rivero et al. 2004). Hence, the improved enzymatic ROS quenching activities helped cv. Arka Vikas to perform better in terms of lower membrane damage and higher water potential compared to IIHR 2195 and Abhinava. From our study it is evident that mild temperature stress caused significant increase in electrolytic leakage, activities of SOD, GR, POX and reduction in water potential and CAT activity across the tomato genotypes tested. Among the genotype cv. Arka Vikas performed better in terms of improved antioxidant enzyme activity, membrane stability and water potential.

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