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Changes in fruit quality and carotenoid profile in tomato (*Solanum lycopersicon* L.) genotypes under elevated temperature

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ABSTRACT

Tomato (*Solanum lycopersicon* L.) is a rich source of carotenoids, especially lycopene, and is affected severely by high temperatures under tropical conditions. To study the effect of elevated temperature on lycopene content and other quality parameters, five tomato genotypes, viz., RF4A, Abhinava, Arka Saurabh, IIHR 2195 and Arka Vikas, were grown in a temperature gradient tunnel (TGT) facility under 33.4 and 35.4ºC temperature conditions. Fruits were analyzed for total carotenoids, total phenols, total flavonoids, total sugars, TSS, acidity, Vitamin C besides carotenoids profile (β-carotene, lycopene, phytoene and luteoxanthin content). Results revealed that all the quality parameters studied were superior at 33.4ºC, compared to 35.4ºC in all the genotypes. 'IIHR 2195' recorded highest total phenols (479.28mg/100g dw), total flavonoids (70.27mg/100g dw), ferric reducing antioxidant potential (FRAP) (310.53mg/100g dw), diphenyl picryl hydrazyl (DPPH) radical (487.89mg/100g dw), Vitamin C content (292.25mg/100g dw) and total sugars (606.88mg/g dw) at 33.4ºC and at 35.4ºC. 'RF4A' and 'Arka Vikas' were found to have better total carotenoids content and lycopene at higher temperature than other genotypes. 'Arka Vikas' recorded highest total soluble solids (TSS) (8.9ºBrix) and acidity (0.80%) at 35.4ºC. Higher TSS and acidity were recorded at 35.4ºC than at 33.4ºC in all the five genotypes. Genotypic variation was observed in the above stated biochemical parameters in response to elevated temperatures.

Key words: Tomato, TGT, antioxidants, elevated temperature, UPLC

INTRODUCTION

Global warming is an important issue threatening most horticultural crops, and can lead to serious consequences in food production. Tomato, being sensitive to temperature, is likely to be influenced by elevated temperatures under a climate change scenario (Laxman *et al*, 2013). Increase in temperature under climate-change circumstances affects crop yield, in turn affecting sustained supply for meeting a growing demand.

Tomato, an important horticultural crop in India, is currently the second largest vegetable in terms of production. It is one of the most consumed vegetables in the world. Tomatoes are rich in bioactive compounds, including carotenoids (lycopene, β-carotene, phytoene and luteoxanthin), ascorbic acid, flavonoids and phenolic compounds (Kaur *et al*, 2013). Along with phenols, higher intake of flavonoids, Vitamin C and carotenoids has been reported to reduce the risk of many degenerative diseases (Agarwal and Rao, 2000).

Optimal mean daily temperatures for tomato lie between 21 and 24°C, depending on the developmental stage (Geisenberg and Stewart, 1986). Supra-optimal temperatures cause a series of complex morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang *et al*, 2003). Temperature has a significant influence on many aspects of growth and development in tomato. Temperature below 16ºC can cause flower abscission, while temperature above 30ºC can cause fruit cracking and blotchy ripening (Islam, 2011). Impact of high temperature on the plant is not limited to flowering and fruit-set, but also subsequent development and maturity of the fruit, and fruit quality. Lee and Kader (2000) reported higher Vitamin C content in tomato grown under low temperatures than that under high temperature. High temperature also affects biosynthesis of carotenoids,

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especially lycopene (Kaur *et al*, 2013). Environmental factors other than temperature, like, plant nutrition and light, can also considerably affect biosynthesis of carotenoids. Phenolic acids and flavonols are reported to increase under high temperature conditions in strawberry (Wang and Zheng, 2001). Although sufficient literature is available on fruit quality parameters in different tomato genotypes, studies on varietal response to elevated temperature in terms of fruit quality are scanty and this information is essential to identify varieties suited to a changing climate. Therefore, the present experiment was set in a temperature gradient tunnel to study the effect of temperature on fruit quality parameters and carotenoid profile in five tomato genotypes.

MATERIAL AND METHODS

The experiment was carried out at ICAR-Indian Institute of Horticultural Research, Bengaluru, in a temperature gradient tunnel during the months of October 2011 to February 2012. Bengaluru is located at 13º58' N latitude, 78ºE longitude and 890m above mean sea level. Five genotypes of tomato (*Solanum lycopersicon* L.), viz., RF4A, Abhinava, Arka Saurabh, IIHR 2195 and Arka Vikas, were selected for the study. Twenty-five day old seedlings were transplanted into 20 litre capacity plastic containers filled with soil, FYM and sand, in the ratio of 2:1:1. Temperature gradient tunnel (TGT) measuring 18m length, 4.5m width and 3m height, covered with a polycarbonate sheet was used in the study. One week after transplanting, the containers were shifted to TGT for imposition of temperature treatments. One set comprising six plants each of the five genotypes was placed near the cooling pad and another set with the same number of plants was placed towards the fan (where the average air temperature was about 2°C higher than at the cooling-pad end). Daily temperatures and relative humidity (RH) during fruit growth period recorded inside TGT are shown in Fig. 1. The gradient inside TGT was maintained only during daytime, as TGT worked on the pad-and-fan system. Since there was no gradient in the night-time minimum temperature, only one value for temperature is indicated (Fig. 1). Photosynthetically active radiation (PAR) inside the TGT was about 85% of that of the light outside. The plants were provided with recommended dose of fertilizer, and suitable crop protection measures were applied when required.

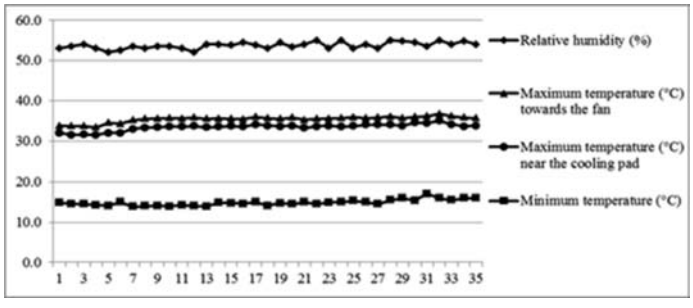

Fig. 1. Daily maximum/minimum temperature (°C) and relative humidity (%) during the last 35 days of fruiting season

Freshly-harvested, fully ripe fruits were used for analysis. Fruits were crushed in a blender and a known quantity of the homogeneous mass was set apart for analysis. Quality parameters like TSS, % acidity, Vitamin C content, total phenols, total flavonoids, FRAP, DPPH, total carotenoids and total sugars were analyzed.

Total soluble solids (TSS) were recorded using a digital refractometer (ARKO India Ltd., Model DG-NXT) and expressed in ºBrix. Acidity was determined by the titration method (AOAC, 942.15) using phenolphthalein as the indicator. Acidity was expressed in per cent citric acid equivalent. Vitamin C content was determined using 2,6-dichlorophenol indophenol (DCPIP) method (AOAC, 967.21) and calculated as mg ascorbic acid equivalent per 100g dry weight. Total phenols present in 80% methanol extract were estimated by Folin-ciocalteu method (Singleton and Rossi, 1965). Methanol extract was mixed with FCR reagent and the color developed with 20% sodium carbonate reagent. Intensity of color developed was read at 700nm using a spectrophotometer (T80+ UV/VIS Spectrophotometer, PG Instruments Ltd., UK). Results were expressed in mg gallic acid equivalent per 100g dry weight. Total flavonoids content was estimated as per Chun *et al* (2003). Flavonoids present in the 80% methanol extract were estimated using 5% $NaNO_2$ and 10% $AlCl_3$. Absorbance of the pink mixture was read at 510nm and expressed as mg catechin equivalent per 100g dry weight. Antioxidant capacity was measured as FRAP, using a modified method of Benzie and Strain (1996). Methanol extract (0.2ml) was mixed with 1.8ml FRAP reagent. Intensity of the blue colour that developed was measured at 593nm. Total antioxidant capacity (as ferric reducing antioxidant potential) was calculated and the antioxidant capacity was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) per 100g dry weight. Radical-scavenging ability was measured using DPPH radical assay of Kang and Saltveit (2002). A 0.2ml aliquot of methanol extract was mixed with 0.3ml of 10mM acetate buffer (pH 5.5) and 2.5ml methanolic 0.2mM DPPH solution. Reduction in color due to scavenging of DPPH radicals by antioxidants was estimated by reading the absorbance at 517nm. Radical-scavenging ability was expressed as weight of the sample required for 50%

reduction in DPPH radicals. Total sugars present in the 80% ethanol extract were estimated using dinitrosalicylic acid method (Miller, 1959). A 0.2ml aliquot of extract was mixed with DNS reagent and the absorbance read at 540nm, expressed as mg glucose equivalent per gram dry weight using a standard curve. Total carotenoids and lycopene content were analyzed by spectrophotometric method (Lichtenthaler, 1987). Carotenoids were estimated by extracting with acetone, partitioned to hexane, and their absorbance read at 470 and 503nm. Standards were used for calibration, and results were expressed as mg per 100g dry weight.

Carotenoid profile by UPLC

Carotenoid profile was estimated by UPLC as per Serino *et al* (2009) with minor modifications. Acquity-UPLC system from Waters (Milford, MA, USA) consisting of a quaternary pump, auto sampler injector and PDA detector equipped with Acquity-UPLC BEH-C18 column (1.7µm, 2.1X50mm) with BEH-C18 (1.7µm, 2.1X5mm) guard column was used. Instrument control and data processing were done using Mass Lynx software. The mobile phase consisted of phase-A acetonitrile:methanol:ethyl acetate (53:7:40) and phase-B methanol. Isocratic flow rate of 0.2ml/min was used in the ratio of A:B (95:5) for 6 min with PDA scanning from 200 to 650nm. Individual carotenoids were identified by diode array spectral characteristics, retention time and relative elution order compared to standards and values in literature. Carotenoids were quantified as β-carotene equivalents. A known quantity (1ml) of hexane extract was evaporated to dryness, and residual carotenoids were dissolved in the mobile phase and filtered through 0.2µm nylon filter prior to ion injecting in UPLC for further analysis. The detection was monitored at 450nm for β-carotene, 470nm for lycopene, 286nm for phytoene and 420nm for luteoxanthin.

Statistical analysis

Data were subjected to Analysis of Variance using ANOVA, and, means were compared, with critical difference at $P \leq 0.05$.

RESULTS AND DISCUSSION

Changes in fruit quality parameters in five tomato genotypes at two temperature conditions are presented in Table 1. TSS increased with increase in temperature in all the genotypes (5.6 to 7.2ºBrix) and ranged from 3.8 to 7.1ºBrix at 33.4ºC, and at 35.4ºC, it ranged from 4.5 to 8.9ºBrix. Similar trend was observed in per cent acidity too. Acidity ranged from 0.34 to 0.68% at 33.4ºC, whereas, at 35.4ºC, the acidity ranged from 0.39 to 0.80%. Sugars and acids are important components in tomato fruit flavor (George *et al*, 2004; Kaur *et al*, 2013). Increase in titratable acidity with increase in temperature has been reported (Khanal, 2012). Among genotypes, Arka Vikas recorded the highest TSS (8.9ºBrix) and acidity (0.80%) at 35.4ºC. Higher temperature is known to enhance soluble solids level in relation to ambient temperature conditions (Gunawardhana and De Silva, 2011; Khanal, 2012).

Table 1. Changes in fruit quality parameters of five tomato genotypes at two temperature conditions

Genotype	Mean temperature during fruiting stage	TSS (ºBrix)	Acidity (%)	Vitamin C (mg/100g dw)	Total phenols (mg/100g dw)	Total flavonoids (mg/100g dw)	FRAP (mg/100g dw)	DPPH (mg/100g dw)	Total carotenoids (mg/100g dw)	Total sugars (mg/g dw)
RF4A	33.4ºC	5.9	0.53	265.68	344.51	44.63	209.34	321.56	270.36	375.91
	35.4ºC	7.1	0.66	272.49	378.99	45.06	160.37	306.32	191.97	366.21
Abhinava	33.4ºC	6.1	0.46	272.06	361.73	42.83	202.06	310.16	228.98	423.03
	35.4ºC	8.6	0.66	263.70	377.48	43.21	168.35	293.08	136.14	221.03
Arka Saurabh	33.4ºC	3.8	0.34	225.94	336.88	33.03	198.39	315.28	269.14	403.99
	35.4ºC	4.5	0.39	258.59	419.68	45.52	191.93	318.73	176.74	264.38
IIHR 2195	33.4ºC	4.9	0.46	292.25	479.28	70.27	310.53	487.89	205.13	606.88
	35.4ºC	6.8	0.64	271.63	461.61	66.88	231.92	415.20	155.45	379.79
Arka Vikas	33.4ºC	7.1	0.68	226.19	352.64	49.66	208.72	343.55	197.33	347.23
	35.4ºC	8.9	0.80	212.87	436.62	64.40	192.90	377.65	158.24	254.68
Mean	33.4ºC	5.6	0.49	256.42	375.01	48.08	225.81	355.69	234.19	431.41
	35.4ºC	7.2	0.63	255.86	414.88	53.01	189.09	342.20	163.71	297.22
CD for varieties (V) ($P \leq 0.05$)		0.02	0.01	1.98	1.67	0.82	1.36	1.40	0.81	1.86
CD for temperature (T) ($P \leq 0.05$)		0.01	0.01	NS	0.67	0.33	0.55	0.56	0.32	0.74
CD for V x T ($P \leq 0.05$)		0.05	0.03	3.96	3.34	1.64	2.73	2.80	1.62	3.72

NS= Non-Significant

Vitamin C content did not show significant differences among the two temperature treatments. However, among genotypes, IIHR 2195 and RF4A recorded higher Vitamin C content at 33.4ºC and 35.4ºC respectively compared to other genotypes. Total phenols and flavonoids increased with increase in temperature in all the genotypes (375.01 to 414.88 and 48.08 to 53.01 mg/100g dw for total phenols and total flavonoids respectively). Higher total phenols and flavonoids were observed in cv. IIHR 2195. Phenolic substances are reported to have a protective effect on ascorbic acid (Toor and Savage, 2006). Therefore, presence of phenolics and flavonoids in tomato cells may have helped maintain ascorbic acid level. Ferreyra *et al* (2007) also reported ascorbic acid level to be not significantly affected by temperature during the growth season. Wang and Zheng (2001) found elevated growth temperature of 30ºC to significantly enhance the content of phenolic acids and flavonols in strawberry. Accumulation of phenolics at higher growth temperature has been reported in other crops too (Wang, 2006; Toor *et al*, 2006).

Total antioxidant capacity and radical-scavenging ability were assessed using FRAP and DPPH methods respectively. All the genotypes recorded significantly higher FRAP and DPPH at 33.4ºC. Among genotypes, higher FRAP and DPPH values were recorded in IIHR 2195 at both 33.4ºC and 35.4ºC. 'RF4A' and 'Abhinava' recorded lower FRAP values at 35.4ºC compared to other genotypes. 'Abhinava' recorded lower DPPH values at both 33.4ºC and 35.4ºC.

All the genotypes recorded higher total sugars at 33.4ºC than at 35.4ºC. IIHR 2195 recorded the highest total sugars (606.88mg/g dw) at 33.4ºC. Temperature changes are known to affect fruit maturation and growth through influencing regulation of the enzymes acid invertase and sucrose synthase or cell-expansion and division and regulation of sugar transport into the fruit (Fleisher *et al*, 2006). Gautier *et al* (2005) reported decrease in sugar content in cherry tomato when fruit temperatures increased. Sugar content in purple passionfruit juice was highest at low growth temperature, and lowest at high growth temperature. More sucrose accumulated at low temperature, while glucose and fructose content increased at higher temperature (Utsunomiya, 1992). All these studies support our observations in tomato.

In the present study, all the genotypes studied recorded higher total carotenoids and lycopene content at 33.4ºC than at 35.4ºC. Carotenoid profiles indicated that β-carotene, lycopene, phytoene and luteoxanthin content was greater at 33.4ºC in all genotypes. Temperature had a significant influence on total carotenoids and lycopene content. High temperature may lead to degradation of lycopene (Demiray *et al*, 2013), in addition to a reduced synthesis (Helyes *et al*, 2007). Temperatures greater than 30ºC lead to inhibition of lycopene synthesis in normal red cultivars of tomato and synthesis is restored when the temperature drops below 30ºC. These temperature effects vary with the tomato cultivar (Garcia and Barrett, 2006). Temperatures below 12ºC strongly inhibit lycopene biosynthesis, while temperatures over 32ºC stop this process altogether (Dumas *et al*, 2003). Temperature during fruit ripening plays a more important role in lycopene biosynthesis than it does during fruit growth period. Fig. 2 shows chromatograms of tomato carotenoids at 33.4ºC and 35.4ºC. All the carotenoid pigments under study diminished at 35.4ºC in all five genotypes (Table 2). Greatest reduction was observed in two major pigments, lycopene and phytoene. However, reduction was lower in 'RF4A' and 'Arka Vikas'. Higher reduction was noticed in 'Abhinava'.

In conclusion, Changes in fruit quality parameters in five tomato genotypes under elevated temperature were studied under TGT. Variations were observed among tomato genotypes for fruit quality parameters at elevated temperature. Increase in temperature improved TSS and per cent acidity, but decreased total carotenoids, lycopene

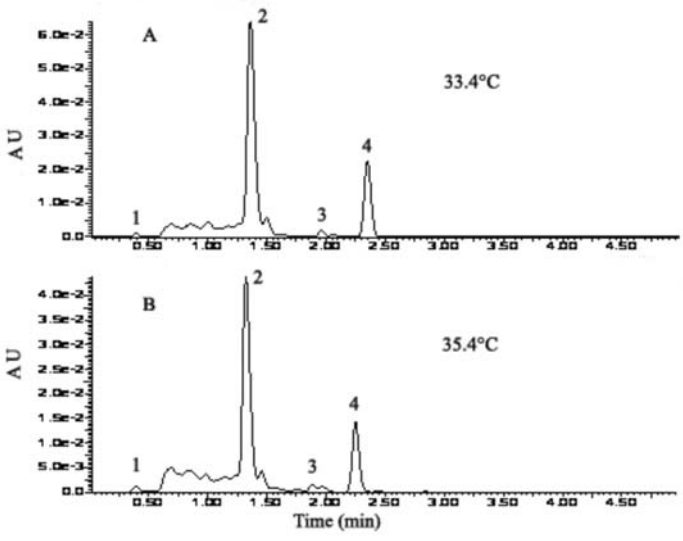

Fig. 2. UPLC chromatograms of carotenoids in tomato fruit extract. Chromatogram A represents carotenoid profiling at 33.4°C (maximum temperature near the cooling pad) and chromatogram B represents carotenoid profiling at 35.4°C (maximum temperature towards the fan). Peaks identified are: (1) Luteoxanthin, (2) Lycopene, (3) β-Carotene and (4) Phytoene

Table 2. UPLC data on phytoene, lycopene, β-carotene and luteoxanthin in five tomato genotypes at two different temperature conditions

Genotype	Phytoene (mg/100g dw)			Lycopene (mg/100g dw)			β-Carotene (mg/100g dw)			Luteoxanthin (mg/100g dw)		
	33.4ºC	35.4ºC	% increase/ decrease over 33.4ºC	33.4ºC	35.4ºC	% increase/ decrease over 33.4ºC	33.4ºC	35.4ºC	% increase/ decrease over 33.4ºC	33.4ºC	35.4ºC	% increase/ decrease over 33.4ºC
RF4A	29.20	27.05	-7.35	174.38	130.43	-25.20	11.38	5.95	-47.70	4.48	4.94	10.36
Abhinava	67.02	35.10	-47.64	150.65	88.91	-40.98	8.11	6.20	-23.52	3.88	2.17	-44.16
Arka Saurabh	29.74	17.67	-40.58	175.54	121.34	-30.87	5.74	3.67	-36.09	5.01	2.95	-41.20
IIHR 2195	36.67	19.25	-47.49	131.46	88.38	-32.77	7.69	3.69	-52.06	4.03	2.23	-44.57
Arka Vikas	30.08	17.64	-41.37	146.46	122.61	-16.29	4.39	5.22	18.67	3.43	1.61	-53.19
Mean	38.54	23.34		170.78	122.65		7.46	4.94		4.17	2.78	
CD for varieties (V) (*P≤0.05*)	0.40			2.68			0.46			0.13		
CD for temperature (T) (*P≤0.05*)	0.16			1.07			0.18			0.05		
CD for V x T (*P≤0.05*)	0.80			5.36			0.92			0.27		

and total sugars in all the genotypes studied. Among the genotypes, IIHR 2195 was found to be better in terms of total phenols, total flavonoids, FRAP, DPPH and total sugars at 33.4ºC, as also at 35.4ºC. 'Arka Vikas' was found to be high in TSS and per cent acidity at 33.4ºC. RF4A and Arka Vikas were found to be good at maintaining lycopene level at elevated temperature, compared to the other genotypes. Genotype IIHR 2195 is recommended for cultivation at elevated temperatures.

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