Response of Antioxidant System to Postharvest Salicylic Acid Treatment in Tomato (*Solanum lycopersicum* L.) Fruit Stored at Ambient Temperature

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ABSTRACT

Tomato fruit (cultivars Hisar Arun and BSS-488) harvested at turning stage were treated with salicylic acid and evaluated for physicochemical traits and antioxidant system during storage at ambient temperature. The increase in the physiological loss in weight, lycopene and β -carotene content were significantly delayed by salicylic acid treatment and delayed the decrease in fruit firmness for both tomato cultivars. Compared with the control fruits, salicylic acid treatment significantly altered activities of SOD, catalase, peroxidase, ascorbate peroxidase, and lipooxygenase enzymes, whereas delay in increase in the MDA content and H₂O₂ content was observed during storage period. Our results revealed potential of salicylic acid treatment on tomato fruits in delaying biochemical changes and amelioration of oxidative damage during storage. The exogenous application of salicylic acid may thus be an effective approach in enhancing the quality characteristics and antioxidant potential of tomato fruit stored at ambient conditions.

Keywords: Biochemical changes, Postharvest losses, Quality characteristics.

INTRODUCTION

Tomato (Solanum lycopersicum L.) is a member of Solanaceae family and a widely consumed vegetable fruit. India ranks second in the production of total fruit as well as in tomato fruit production. Tomato is enriched with several minerals, vitamins, and pigments, and among pigments, lycopene constitutes the major part of tomato, which imparts red color to it during ripening (Helyes *et al.*, 2009). Fruit color, flavor, appearance and texture determine the quality of fruit. Tomato comes under the category of perishable fruits; hence, it is prone to huge loss during processing and transportation. As a result, fruit becomes dull and wilted, which results in reduced market value, causing economic losses to farmers. Almost 25% of fruit is wasted because of improper post-harvest handling and storage conditions. Also, different environmental conditions during transportation such as light, temperature, and dark conditions, can also alter the plant defense responses and growth metabolism (Poór et al., 2018). A number of methods

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such as application of edible coatings *i.e.* Chitosan, melatonin, salicylic acid etc., has been studied to prevent the postharvest losses (Kumar et al., 2018; Sultana et al., 2019; Zhang et al., 2020). Coatings are usually environmentally friendly and safe for human consumptions as they are made of natural ingredients.

Salicylic (SA) is a phenolic Acid compound acting as a plant growth modulator by regulating complex physiological processes in plants. It has high potential in monitoring post-harvest losses as a natural and safe phenolic compound and is suggested to be safe according to Food and Drug Administration (FDA). Previous studies on coating of SA suggest that it plays an important role in delaying ripening processes of fruits by inhibiting the production of ethylene (Abbasi et al., 2009; Peyro et al., 2017; Tareen et al., 2012; Valero et al., 2011). Our previous work also suggest that SA coating on tomato fruits not only delayed ripening processes but efficiently inhibited the peak values of ethylene (Kumar et al., 2018).

Several biochemical changes take place during ripening such as change in phenolics, colour, flavor, texture, chlorophyll and dissolution of polysaccharides to simple sugars (Phan et al., 1973). These changes are results of production of Hydrogen peroxide (H_2O_2) , Oxide radicals (O_2) and Reactive Oxygen Species (ROS) accumulation in the process of ripening. These reactive oxygen species are controlled by plant's antioxidative defense system, so, the changes are noticed during ripening in both reactive species and antioxidants level. The level of the antioxidative enzymes, such as, Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POX) and Ascorbate Peroxidase (APX) and Metabolite Changes [Malonaldehyde (MDA) and Lipoxygenase (LOX)] during storage conditions affects the fruit's shelf-life. SA treatment elicits induction of antioxidative defense system (Ghasemzadeh and Jaafar, 2013), and the fruit treated with SA enhances the activity of these enzymes (Manochehrifar, 2010) by

maintaining oxidative damage (Rao et al., 1997) and increases plant tolerance to postharvest stresses (Senaratna et al., 2000). Thus, their quantification in tomato fruit during storage and SA application could reveal their role during ripening of fruit. Therefore, the present study was planned to evaluate the effect of salicylic acid on the shelf life of tomato fruit by regulating antioxidative defense machinery.

MATERIALS AND METHODS

Chemicals

All the chemicals used in study were procured from Bio-Rad, Sigma-Aldrich Chemical Co., Ambion: Life Technologies, Hi-Media Laboratories, Thermo Scientific and SRL.

Plant Material

Two varieties of tomato fruit (Hisar Arun and BSS-488) at turning stage were procured from the farms of Department of Vegetable Sciences, CCS Haryana Agricultural University, Hisar, Haryana, India. The experimental work was carried out in the Department of Biochemistry, CCS Haryana Agricultural University, Hisar, Haryana, India. The fruits selected for study had uniform colour, shape and size. Upon sorting, fruits were gently washed with tap water containing 2% (w/v)sodium hypochlorite solution, rinsed with double distilled water and dried. Dried fruit were divided into two groups and used for the salicylic acid treatment. The salicylic acid solution (0.75 mM) was prepared according to our previous work Kumar et al. (2021) and the treated fruits were stored for 12 days separately in boxes (50×33×28 cm; 90 fruits/box) at ambient temperature $(25\pm1^{\circ}C)$ and relative humidity (75±5%). Three fruit from each box were used periodically for analysis of all parameters at 3-day intervals.

Physiological Parameters

The weight of freshly harvested fruit was recorded at the time of harvesting (0 d of storage) and termed as initial weight. On each day of observation, the stored fruits were again weighed and termed as final weight. The Percent Loss in Weight (PLW) on each sampling date was calculated using the following formula:

PLW=	Initial weight-Final weight ×100
	Initial weight

The PLW during storage period was compared with that of the preceding storage period to have total PLW on that day of storage.

Flesh firmness was measured by using hand-held penetrometer (Biogen Fruit pressure tester BGS-25), using cylindrical plunger of 8 mm diameter and firmness scale of 13 N. The firmness was measured from each side of the equatorial region of the fruit and expressed in N (Kumar *et al.*, 2021).

Antioxidative Metabolites Estimation

The lycopene content was estimated by the method of Darsan *et al.* (2013). Tomato fruit tissue was homogenized with hexane: ethanol: acetone in 2:1:1 ratio and incubated in bright light for 1 hour. The suspension was vortexed and, after 10 minutes, phases were separated. The absorbance of the upper layers was recorded at 503 nm in UV-VIS double beam spectrophotometer (Specord 210 plus, AnalytikJena, Germany).

 β -carotene content was determined as described by AOAC (2000). The fresh fruit sample was homogenized in watersaturated n-butanol to make a suspension and incubated for 16 h at room temperature in dark. The suspension was vortexed and filtered through Whatman filter paper No. 1. The absorbance of the filtrate was recorded at 440 nm. The amount of β -carotene was calculated from the standard calibration curve of β -carotene (0.5-5.0 µg).

Antioxidative Enzymes

Extraction

The fresh tissue (1 g) was homogenized in 10 mL 0.1M phosphate buffer (pH 7.0) containing 1% insoluble polyvinylpyrolidine. The homogenized sample was centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was decanted and used for estimation of antioxidative enzymes.

Superoxide Dismutase

SOD activity was estimated by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium using modified method of Giannopolitis and Ries (1977). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 14 mM L-methionine, 10 µM nitroblue tetrazolium, 3 µM riboflavin, 0.1 mM EDTA and enzyme extract. The reaction mixture was then placed under light source consisting of two 15 W-fluorescent lamps (Phillips, India). The absorbance was measured at 560 nm. One enzyme unit was defined as the amount of enzyme that could cause 50% inhibition of the photochemical reaction.

Peroxidase

POX activity was estimated by the method of Shannon *et al.* (1966). The reaction mixture (2.75 mL) contained 50 mM phosphate buffer (pH 6.5), 0.5% H₂O₂ and 0.2% *O*-dianisidine and enzyme extract. The reaction was initiated by the addition of H₂O₂. The change in absorbance was read at 430 nm for an interval of 3 minutes. One unit of peroxidase was defined as the amount of enzyme required to cause change in absorbance per min under assay condition.

Catalase

CAT activity was determined by the method of Sinha (1972). The reaction mixture (1.0 mL) consisted of 0.1M phosphate buffer (pH 7.0), 0.2 M hydrogen peroxide and diluted enzyme extract. The reaction mixture was incubated at 37°C for 3 minutes and the reaction was terminated by adding 5% (w/v) potassium dichromate and glacial acetic acid (1:3 v/v). The mixture was heated in boiling water bath for up to 10 min. A control was run under similar conditions where enzyme extract was added after termination of the reaction. The absorbance was measured at 570 nm. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the oxidation of 1 µmole H₂O₂ per minute under assay conditions.

Ascorbate Peroxidase

APX activity was estimated by the method described by Nakano and Asada, (1981). The reaction mixture (2.7 mL) contained 100 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2 and enzyme extract. The decrease in absorbance at 290 nm was recorded, which corresponded to oxidation of ascorbic acid. The enzyme activity was calculated using the molar extinction coefficient of 2.8 mM⁻¹ cm⁻¹ for ascorbic acid.

Oxidative Indices Estimation

Lipoxygenase

LOX activity was determined by the method of Suurmeijer *et al.* (1998). The reaction mixture contained 30 mM linoleic acid solution in methanol, 100 mM phosphate buffer (pH 6.8) and enzyme extract. Increase

in absorbance was measured at 234 nm for an interval of 2 minutes. One enzyme unit was defined as the amount of enzyme causing 0.1 unit change in Optical Density (OD) at 234 \min^{-1} .

Malondialdehyde

MDA content was determined by the method of Heath and Packer (1968). To the enzyme extract, 20% (w/v) Trichloroacetic Acid (TCA) containing 0.5 % thiobarbituric acid was added and heated at 95°C for 30 minutes. The mixture after boiling was quickly cooled in ice bath. The absorbance was recorded at 532 nm and the value of non-specific absorption at 600 nm was subtracted from it. The concentration of MDA was calculated using the extinction coefficient of 155 mM⁻¹ cm⁻¹.

Hydrogen Peroxide

 H_2O_2 was estimated by the method of Sinha (1972). To 0.4 mL extract, 0.6 mL of 0.1M phosphate buffer (pH 7.0) and 3 mL mixture of 5 % (w/v) potassium dichromate and glacial acetic acid (1:3, v/v) was added. The mixture was heated for 10 minutes in a boiling water bath. The colour of solution changed to green due to the formation of chromic acetate. After cooling, absorbance was recorded at 570 nm against the reagent blank without sample extract. The quantity of H_2O_2 was determined from the standard curve (10-160 µmoles) of H_2O_2 .

Gaseous Exchange Studies

Three fresh tomato fruits were placed in glass jar fitted with rubber septum and incubated for 4 hours at room temperature. Gas sample was removed from the jars with the help of syringe and injected into a Thermo scientific trace GC 600 gas liquid chromatograph equipped with a Porapak–N column having flame-ionization detector. The temperature of the oven was fixed at 80°C and detector and injector were fixed at 110°C and the flow rate each of N₂, H₂ and O₂ was kept at 1 N. Ethylene identification was based on the retention time compared with standard C₂H₄ (purity 99.9%). The content of ethylene evolved was calculated as described by Hardy *et al.* (1968), and results were expressed as nmol C₂H₄ produced g⁻¹ h⁻¹.

Statistical Analysis

A Completely Randomized Design (CRD) was performed in the present experiment and all the measurements were analyzed by following three factorial Analysis Of Variance (ANOVA) using SPSS Program v23.0 software (SPSS for Windows, Chicago, IL, USA) where first factor included varieties, second factor included treatments and the third factor included the days of storage. All analyses were performed in triplicate and the mean and standard error were calculated. Differences were considered statistically significant at P < 0.05.

RESULTS

Physiological and Biochemical Parameters

The physiological weight loss is mainly due to the increased physiological processes such as transpiration and respiration during storage. There was a progressive increase in the physiological loss in weight (PLW) in both varieties (Table 1). PLW increased significantly (P < 0.05) and progressively with storage time up to 12 DAH in the control fruits of both varieties *i.e.* 3.58% at 3 DAH to 20.11% at 12 DAH in case of 'Hisar Arun' and from 5.85% at 3 DAH to 19.70% at 12 DAH in case of 'BSS-488'. When tomato fruits were treated with salicylic although PLW acid, the increased progressively with increase in the storage

time from 3 DAH to 12 DAH in both varieties, the increase was less as compared to the control fruits. SA treated fruits showed the delay in PLW by 31% in case of 'Hisar Arun' and 19% in case of 'BSS-488' as compared to the control at 12 DAH.

The firmness decreased progressively in the control as well as in SA treated fruits of both varieties with increase in the days of storage. The firmness of control fruits decreased significantly (P< 0.05) from 8.10 N at 0 DAH to 2.13 N at 12 DAH in variety 'Hisar Arun' and from 8.37 N at 0 DAH to 2.50 N at 12 DAH in 'BSS-488' (Table 1). SA treated fruits exhibited significantly (P< 0.05) less decrease in the firmness as compared to the control fruits of both varieties. The delay in decrease in firmness for variety 'Hisar Arun' was about 19% and for variety 'BSS-488'was about 33% at 12 DAH.

A progressive increase in the lycopene and β -carotene (Table 1) content of tomato fruit for both varieties were observed during the storage period. The lycopene content in the control tomato fruits stored at turning stage increased progressively from 0.88 g kg⁻¹ at 0 DAH to 5.05 g kg⁻¹ at 12 DAH in variety Hisar Arun and from 0.77 g kg⁻¹ at 0 DAH to 3.9 g kg⁻¹ at 12 DAH in variety BSS-488. SA treated fruits exhibited delayed increase in the lycopene content, which was approx. 20% at 12 DAH in variety Hisar Arun and approx. 15 % at 12 DAH in variety BSS-488 when compared to the control fruits.

The β -carotene content of the control fruit increased progressively from 0.57 mg 100 g⁻¹ at 0 DAH to 1.01 mg 100 g⁻¹ at 12 DAH in variety Hisar Arun, and from 1.05 mg 100 g⁻¹ at 0 DAH to 1.51 mg 100 g⁻¹ at 12 DAH in variety BSS-488. The exogenous application of SA significantly affected the degradation of carotene content and resulted in postponement of increase in β -carotene content. It was observed that the delay in increase in β -carotene content in SA treated fruits was about 13% at 12 DAH in Hisar Arun and about 11% in BSS-488.

Variety	DAH	Salicylic acid (mM)	Physiological loss in weight (%)	Fruit Firmness (N)	Lycopene (mg 100 g ⁻¹)	β -carotene (mg 100 g ⁻¹)
Hisar Arun	0	Control	-	$8.10 \pm .12^{BE}$	$0.88 {\pm}.02^{ m AC}$	0.57 ± 8.49^{AB}
		Treatment	-	$8.13 \pm .08^{CE}$	$0.89{\pm}.01^{\mathrm{AA}}$	0.58 ± 5.65^{AA}
	3	Control	$3.58{\pm}.05^{\mathrm{BC}}$	$6.33 \pm .10^{BD}$	$1.88 \pm .03^{\mathrm{BC}}$	$0.66 {\pm} 9.92^{\mathrm{BB}}$
		Treatment	$4.10 \pm .02^{AB}$	$6.10\pm.03^{CD}$	$1.53 \pm .01^{AB}$	$0.62{\pm}3.57^{AB}$
	6	Control	$8.52 \pm .10^{CC}$	$4.40\pm.05^{\mathrm{BC}}$	$2.82{\pm}.03^{\mathrm{CC}}$	$0.76{\pm}8.93^{\mathrm{BC}}$
		Treatment	$8.52 {\pm} .09^{\mathrm{AC}}$	$4.90 \pm .05^{\text{CC}}$	$2.68 \pm .03^{\mathrm{AC}}$	$0.69{\pm}6.78^{ m AC}$
	9	Control	$14.90 \pm .23^{CD}$	$3.13 \pm .05^{\mathrm{BB}}$	$4.79 \pm .08^{\text{CD}}$	0.87 ± 13.66^{BD}
		Treatment	$11.52 \pm .07^{AD}$	$3.40\pm.02^{\mathrm{BC}}$	$3.64 \pm .02^{AD}$	0.77 ± 4.49^{AD}
	12	Control	$20.11 \pm .20^{CE}$	$1.93 \pm .02^{AB}$	$5.05 \pm .05^{\text{CE}}$	1.01 ± 9.95^{BE}
		Treatment	$13.82 \pm .08^{\text{AE}}$	$2.30 \pm .01^{AC}$	$4.03 \pm .02^{AE}$	0.88 ± 5.11^{AE}
BSS-488	0	Control	-	$8.37 \pm .12^{BE}$	$0.77 \pm .01^{AC}$	1.05 ± 15.57^{AB}
		Treatment	-	$8.34 \pm .08^{\text{CE}}$	$0.76 \pm .01^{AA}$	$1.04{\pm}10.36^{AA}$
	3	Control	$5.85{\pm}.09^{\rm BC}$	$7.40 \pm .11^{BD}$	$1.35 \pm .02^{BC}$	1.17 ± 17.51^{BB}
		Treatment	$3.98{\pm}.02^{\mathrm{AB}}$	$7.63 \pm .04^{\text{CD}}$	$1.08{\pm}.01^{\mathrm{AB}}$	$1.14{\pm}6.50^{AB}$
	6	Control	$11.21 \pm .13^{CC}$	$5.77 \pm .07^{\mathrm{BC}}$	$2.18 \pm .03^{CC}$	1.29±15.21 ^{BC}
		Treatment	$7.54{\pm}.07^{\mathrm{AC}}$	$6.33 \pm .06^{CC}$	$2.01 \pm .02^{\mathrm{AC}}$	1.19 ± 12.06^{AC}
	9	Control	$14.84 \pm .23^{CD}$	$3.50\pm.06^{\mathrm{BB}}$	$3.20\pm.05^{\text{CD}}$	1.38 ± 21.68^{BD}
		Treatment	$11.88 \pm .07^{\mathrm{AD}}$	$4.90\pm.03^{\mathrm{BC}}$	$2.73 \pm .02^{\mathrm{AD}}$	1.26 ± 7.81^{AD}
	12	Control	$19.70 \pm .19^{CE}$	$2.50{\pm}.03^{\mathrm{AB}}$	$3.90{\pm}.04^{\text{CE}}$	1.51 ± 14.82^{BE}
		Treatment	$15.92 \pm .09^{AE}$	$3.33 \pm .02^{AC}$	$3.33 \pm .02^{AE}$	1.34 ± 8.33^{AE}

Table 1. Effect of salicylic acid treatment on physiological and biochemical attributes of tomato fruit.

 $^{A-E}$ Values with different superscripts in the same row are significantly different (P< 0.05). Values are expressed on fresh weight basis. The error interval represents standard deviations in measured data. DAH: Days After Harvest.

Antioxidative Enzymes Estimation

The activities of antioxidative enzymes were affected by increase in the storage days for both 'BSS-488' and 'Hisar Arun' tomato fruits. A progressive decrease in SOD activity was observed in the control and SA treated fruits. It decreased from 36.62 Units g^{-1} FW on day 0 to 22.14 Units g^{-1} FW on day 12, while it decreased from 28.11 Units g^{-1} FW on day 0 to 16.86 Units g^{-1} FW on day 12 in the control fruits of 'Hisar Arun' and 'BSS-488', respectively. The per cent reduction in SOD activities of 'Hisar Arun' was 40% on day 12 for the control fruits whereas for 'BSS-488' it was 40% as compared to activity on day 0. The delay in decrease in SOD activity was observed in SA treated fruits. The per cent reduction in SA treated fruits of 'Hisar Arun' was 27% on day 12 whereas for 'BSS-488' it was 21% (Figures 1-C and -D).

Similarly, a progressive decrease in POX activity was observed in the control and SA treated fruits (Figure 3). SA treated fruits showed the delay in decrease in the enzyme activity as compared to the control fruits for both varieties. It was observed that there was about 34% reduction in POX activity of the control fruits for 'Hisar Arun' while the reduction was about 36% for the control fruits of 'BSS-488' on day 12 as compared to the activity on day 0. SA treated fruits showed the postponement in decrease in POX activity, which was about 24% reduction for 'Hisar Arun' fruits and about 25% reduction for 'BSS-488' on day 12 as compared to activity of POX on day 0 (Figures 1-G and -H).

The activities of CAT and APX increased up to 6 DAH in the control fruits of 'Hisar Arun' whereas activities increased up to 9 DAH in the control fruits of 'BSS-488' and declined thereafter for both varieties (Figures 1-A, -B, -E and -F). SA treated

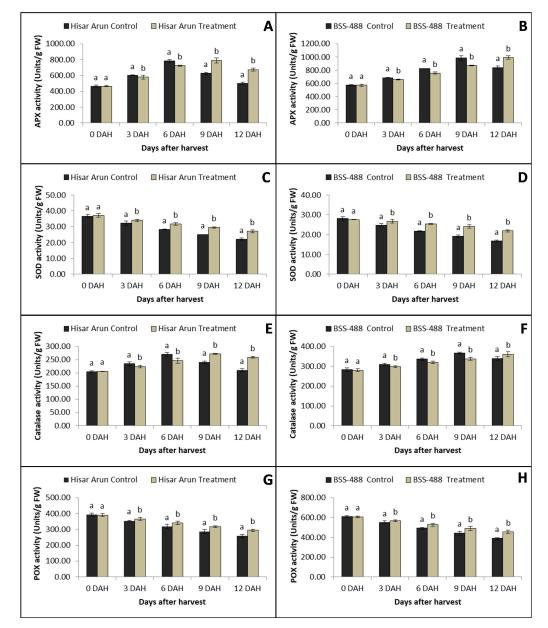


Figure 1. Effect of salicylic acid treatment on Ascorbate peroxidase (A and B), Superoxide dismutase (C and D), Catalase (E and F) and Peroxidase (G and H) activities in 'Hisar Arun' and 'BSS-488' tomato fruits. Values are expressed on fresh weight basis. Bar represents Mean \pm SD. Superscripts in the same column followed by the same letter do not differ significantly (P< 0.05).

fruits showed the delay in increase in the activities of CAT and APX. In SA treated fruits, the activities of CAT and APX increased up to 9 DAH in case of 'Hisar Arun' tomatoes, while it increased up to 12

DAH in case of 'BSS-488' tomatoes. It was observed that maximum activity was delayed by 3 days in both varieties under the influence of SA.

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Activities of LOX increased during storage in the control and SA treated fruits for both varieties. It increased from 263.86 Units g⁻¹ FW on day 0 to 753.61 Units g⁻¹ FW on day 12 in the control fruits of 'Hisar Arun' and from 182.11 Units g⁻¹ FW to 536.31 Units g⁻¹ FW on day 12 in the control fruits of 'BSS-488'. Salicylic acid treatment delayed the increase in activity of LOX and maintained lower activity throughout the storage period. The delay in LOX activity

was about 15% on day 12 in 'Hisar Arun' fruits and about 17% on day 12 in 'BSS-488' fruits as compared to the control fruits (Figure 2).

Antioxidant Metabolites

As shown in Figures 3-A and -B, significantly (P < 0.05) lower MDA content was observed in SA treated fruits in both

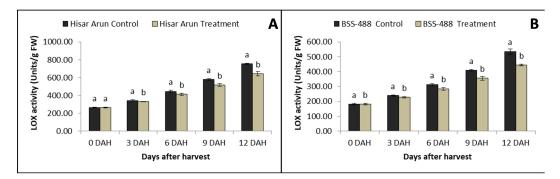


Figure 2. Effect of salicylic acid treatment on LOX activity in 'Hisar Arun' (A) and 'BSS-488' (B) tomato fruits. Values are expressed on fresh weight basis. Bar represents Mean \pm SD. Superscripts in the same column followed by the same letter do not differ significantly (P< 0.05).

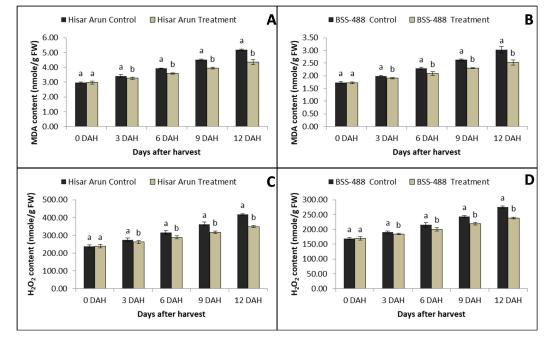


Figure 3. Effect of salicylic acid treatment on MDA (A and B) and H_2O_2 (C and D) content in 'Hisar Arun' and 'BSS-488' tomato fruits. Values are expressed on fresh weight basis. Bar represents Mean±SD. Superscripts in the same column followed by the same letter do not differ significantly (P< 0.05).

varieties as compared to the control fruits. Approximately 16% less MDA content was found on day 12 in 'Hisar Arun' fruits, while about 17% less MDA content was observed on day 12 in case of 'BSS-488' fruits compared with the control fruits. Overall, MDA content increased with increase in the storage days for both varieties.

Similarly, the H_2O_2 content of the control fruits increased with increase in the storage days for both varieties (Figures 3-C and -D). In SA treated fruits, significantly lower (P< 0.05) H_2O_2 content was found in both varieties as compared to the control fruits. About 16% less H_2O_2 content was found on day 12 in 'Hisar Arun' fruits while about 13% less H_2O_2 content was observed on day 12 in case of 'BSS-488' fruits compared with the control fruits.

Ethylene Estimation

Results presented in Figure 4 revealed that the rate of ethylene production first increased up to a specific day and. thereafter, it started declining in both varieties. Significantly higher rate of ethylene production was recorded in variety Hisar Arun as compared to BSS-488. In the control fruits of 'Hisar Arun', the maximum rate of ethylene production were observed at 6 DAH and 9 DAH in case of 'BSS-488' fruits. The delay in the rate of ethylene production was observed for SA treated

fruits for both varieties. In SA treated 'Hisar Arun' fruits, the maximum rate was noticed at 9 DAH instead of 6 DAH and at 12 DAH instead of 9 DAH in case of SA treated 'BSS-488' fruits as compared to the control fruits.

DISCUSSION

Tomato, a perishable crop, deteriorates in quality on storage, which is the major reason for its postharvest loss. This could be prevented by application of shelf-life enhancing compounds such as (MCP), Methylcyclopropene calcium chloride (Aguayo et al., 2006), polyamines (Martinez-Romero et al., 2007) and SA (Kant et al., 2013). In the present study, exogenous application of 0.75 mM SA significantly reduced the deteriorative processes and entities responsible for this process. Along with this, it maintained concentration higher of antioxidative enzymes and metabolites that augment fruit freshness, firmness and quality. There are similar reports in which SA treatments in plums have minimized the softening, chilling injury and color evolution resulting in sustained fruit quality at low temperature in Qingnai (Luo et al., 2011), Santa Rosa (Davarynejad et al., 2015; Sharma and Sharma, 2016) and Satluj (Majeed and Jawandha, 2016) varieties. Similar results have been observed in apricots (Wang et al.,

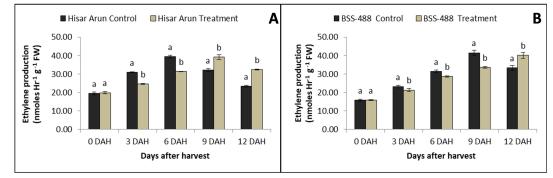


Figure 4. Effect of salicylic acid treatment on ethylene production in 'Hisar Arun' (A) and 'BSS-488' (B) tomato fruits. Values are expressed on fresh weight basis. Bar represents Mean \pm SD. Superscripts in the same column followed by the same letter do not differ significantly (P<0.05).

2015), pomegranate (Sayyari *et al.*, 2011b, 2011a), sweet cherry (Valero *et al.*, 2011), and kiwifruit (Zhang *et al.*, 2003) in which SA treatments minimized the fruit decay against chilling injuries and diseases and improved nutritional content, appearance, quality and texture (Asghari and Aghdam, 2010; Glowacz and Rees, 2016). The fruit firmness was also maintained in plums harvested from SA, MeSA pretreated plants than the control plants derived fruits. Thus, SA helped in maintaining fruit firmness for longer period during storage.

The β -carotene content increased on ripening in tomato fruit during storage. As lycopene is the precursor in β -carotene synthesis, it will also increase during ripening (Su et al., 2015). Similarly, carotenoids, and antioxidant activity, in both hydrophilic and lipophilic compounds were found at higher levels in plums from SA-, ASA-, and MeSA-treated trees than in those from control trees. The postharvest application of SA on sugar apple and mango reduced the lipoxygenase activity as compared to control fruits (Ding et al., 2007; Mo et al., 2008). There are many evidence indicating that exogenous application of SA aid in quality maintenance of fruit and vegetables (Giménez et al., 2016), minimizes disease incidence (Khademi et al., 2012), enhance shelf life (Martínez-2017) Esplá *et al.*, and increases antioxidative potential of fruits (Wang et al., 2015). The results obtained in our experiment showed similarity with tomato (Su et al., 2015), jack fruit (Nhung et al., 2010) and mango (Vásquez-Caicedo et al., 2006).

A number of enzymes take part to overcome the oxidative stress and delay the deteriorative changes to maintain fruit quality. The major enzymes involved in cleansing the reactive oxygen species are CAT, POX, SOD, and APX and defends the cell from oxidative damage. The higher SOD, CAT, and APX enzyme activity have been observed in sugar apple and mango on postharvest SA application against the control fruits (Ding *et al.*, 2007; Mo *et al.*,

2008). In peach fruit also, similar results for antioxidative enzymes have been reported on SA treatment (Tareen et al., 2012), which increased the shelf life of fruit by delaying fruit ripening. Plums harvested from plants pre-treated with MeSA, SA and ASA showed higher carotenoid and antioxidant activity as compared to the control, which proved that SA treatment sustains fruit quality and delays senescence. Similar trend has been reported in sweet cherry where treatment of SA, ASA, or MeSA before higher showed activity of harvest antioxidative enzymes in fruit (Giménez et al., 2017; Valverde et al., 2015), and in peach also, post-harvest SA treatment resulted in increased activity of SOD, POX and CAT enzymes (Tareen et al., 2012). The post-harvest treatment of cucumber with SA alone and in combination with chitosan also increased activity of these enzymes (Zhang et al., 2015). Thus, SA treatment, either post or pre harvest, augment fruit quality maintenance by increasing the antioxidative defense system, which has been reported in many fruit including ber (Ziziphus mauritiana) and guava (Kumar et al., 2014; Mondal et al., 2009), and in our study on tomato. In the present study also, SA application significantly reduced the activities of antioxidant enzymes, which cleanse the reactive species and defend the fruit from senescence and quality degradation.

In climacteric fruits, production of ethylene is considered as the main cause for ripening. In Figure 4, it is observed that ethylene production increased significantly and gradually up to a maximum level and then declined. The results are in accordance with the various investigations on climacteric fruits like tomato (Alexander and Grierson, 2002), banana (Liu et al., 1999), and mango (Sane et al., 2005). During ripening the ethylene production rate increases progressively in all climacteric fruit (Alexander and Grierson, 2002; Burg and Burg, 1965; Lelièvre et al., 1997; Liu et al., 2020). In the present experiment, it was detected that the evolution rate of ethylene

was delayed by SA treatment, which is in agreement with the previous studies (Leslie and Romani, 1988, 1986; Netlak *et al.*, 2021; Kumar *et al.*, 2021). Comparable effect of SA has been documented in various fruits such as, banana (Srivastava and Dwivedi, 2000), strawberry (Babalar *et al.*, 2007), tomato (Kant *et al.*, 2013), kiwifruit (Aghdam *et al.*, 2009; Zhang *et al.*, 2003), and apple (Mo *et al.*, 2008). The ethylene production rate was observed to be higher in variety Hisar Arun compared to BSS-488.

CONCLUSIONS

The results in the present study indicated that SA treated fruits had delayed loss of weight, firmness, lycopene content, carotene content, antioxidative enzymes activities, ethylene production and hence fruit decay. 'BSS-488' variety better responded in the studied traits, compared to 'Hisar Arun'. On the basis of the results obtained in the present studies. SA treatment can be considered effective as an and environmental friendly approach for delaying ripening processes during postharvest storage.

Abbreviations: APX- Ascorbate Peroxidase, CAT- Catalase, LOX-Lipoxygenase, MDA- Malondialdehyde, POX- Peroxidase, SA- Salicylic Acid, SOD-Superoxide Dismutase.

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واکنش سامانه آنتی اکسیدانی به تیمار سالیسیلیک اسید پس از برداشت در میوه گوجه فرنگی (.*Solanum lycopersicum* L) نگهداری شده در دمای محیط

چکیدہ

میوه گوجه فرنگی (کولتیوارهای Hisar Arun **و** BSS-488) که در مرحله رسیدن برداشت شد و با اسید سالیسیلیک تیمار شده بود، در مدت نگهداری در دمای محیط از نظر صفات فیزیکوشیمیایی و سامانه آنتی اکسیدانی مورد ارزیابی قرار گرفت. افزایش تلفات فیزیولوژیکی در وزن، محتوای لیکوین و بتا کاروتن به طور

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قابل توجهی با تیمار اسید سالیسیلیک به تاخیر افتاد و کاهش سفتی میوه را برای هر دو کولتیوار گوجه فرنگی به تاخیر انداخت.در مقایسه با میوه های شاهد، تیمار سالیسیلیک اسید به طور قابل توجهی فعالیت آنزیم های SOD، کاتالاز، پراکسیداز، آسکوربات پراکسیداز و لیپواکسیژناز را تغییر داد، در حالی که تاخیر در افزایش محتوای MDA و محتوای H2O2 در طول دوره نگهداری مشاهده شد. نتایج ما پتانسیل تیمار اسید سالیسیلیک روی میوههای گوجهفرنگی را در به تاخیر انداختن تغییرات بیوشیمیایی و بهبود آسیب اکسیداتیو در طول ذخیرهسازی نشان داد. بنابراین، کاربرد اسید سالیسیلیک ممکن است یک رویکرد موثر در افزایش ویژگی های کیفی و پتانسیل آنتی اکسیدانی میوه گوجه فرنگی ذخیره شده در شرایط محیطی باشد.