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Improvement of Lycopene, Ascorbic Acid and Total Phenol Content of Postharvest Tomato Fruits by Exogenous Application of Salicylic Acid and Methyl Jasmonate

Andleeb Zehra [1*], Manish Kumar Dubey [1], Mukesh Meena [1], Mohd. Aamir [1], Laxmi Ahirwal [2] and R.S. Upadhyay [1]

[1] Laboratory of Mycopathology and Microbial Technology, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi- 221 005, Uttar Pradesh, India
[2] Department of Botany, School of Biological Sciences, Dr. H.S. Gour University, Sagar- 470 003, Madhya Pradesh, India

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Abstract: Biochemical features of tomatoes were evaluated with dip-treatment of chemical inducers such as salicylic acid (SA) and methyl jasmonate (MeJA). Mature tomato fruits (*Lycopersicon esculentum* Mill.) were dipped in 1 mM SA and MeJA solutions along with double-distilled water (control) at 20°C for 15 min. The contents of lycopene, ascorbic acid and total phenolics were measured at 5, 10, 15 and 20 days after treatments. It was observed that SA and MeJA dip treatment enhanced the biosynthesis of these biochemicals with respect to control. Maximum content of all these biochemicals was found in SA treated fruits. Increase in the biosynthesis of these biochemicals is directly related to the yield and quality of fruits.

Key words: Biochemical characterization, Tomato fruits, Lycopene, Ascorbic acid, Total phenols.

Introduction

Tomato (*Lycopersicon esculentum*, Mill.) is very well know vegetable and fruit world-wide due to its significant nutritional importance, taste and color. According to recent FAO (Food and Agricultural Organization) statistics, approximately 160 million tonnes tomatoes are produced annually on 4.7 million hectares of land [1]. Tomato and its products are rich in antioxidants and considered to be a good source of carotenoids, particularly lycopene and β-carotene, ascorbic acid and other phenolic compounds [2]. Lycopene, an important phytochemical present in tomato, which is well known for its coloring effect in tomatoes along with its significant potential to serve as a remarkable antioxidant molecule. Free radicals which cause oxidative damage in body have proven to play a significant role in the emergence of various chronic diseases including cancer, ageing, and cardiovascular disorders [3]. Lycopene as a precursor of β-carotene which a fat-soluble carotenoid exhibit two-fold higher antioxidant activity than β-carotene [4]. Long-chain conjugated double bonds (polyene chains) with an ability to quench free radicals and present in lycopene mainly attribute to its potential antioxidant ability [5]. Other important assigned functions imparted by lycopene include cell signalling and communications [6] modulation of hormonal and immune response, and role in metabolic pathways [7] which may provide additional beneficial effects [8].

Contents of ascorbic acid (AsA) in tomatoes reduce oxidative stress and are mainly attributed to the quality of harvested fruit products as retention of AsA contents in fruits is associated with increased shelf-life [9]. Tomato rich in AsA has

*Corresponding author (Andleeb Zehra)
E-mail: < zandleeb143@gmail.com >

advantages for consumption as a raw in different processed products. Beside AsA, riped tomato fruits are enriched with high content of phenolic and about 98 % of total phenolic are present in the peel of the fruits as flavonols [10,11] with antioxidant potential which have the capacity to enhance the antioxidant properties of carotenoid [12].

Salicylic acid (SA), jasmonic acid (JA) and methyl jasmonate (MeJA) which serve as signal molecules are endogenous plant growth products which have significant role in the growth and development of plants as well as serve as responders to environmental stresses [13]. SA and JA both play a key role in plant resistance [14]. Exogenous application of SA increases resistance to fungal pathogens in sweet cherry [15] and grape berries [16]. It has been reported that fruits viz., apple [17], tomato [18], etc. treated with SA and MeJA during ripening prolonged ripening and extended shelf-life. The main aim of this study was to determine the effect of exogenous application of SA and MeJA on the lycopene, AsA and total phenol content in fruits of tomato. Hence, this study was aimed to explore influence of exogenous application of SA and MeJA on physicochemical properties of tomato fruits.

Material and methods

Experimental design

Fruits were surface sterilized with 2 % (v/v) sodium hypochlorite for 2 min at ambient room temperature and washed thoroughly with deionized water. The fruits were dipped for 15 min in 0.5, 0.75, 1 and 2 mM SA and MeJA solution as inducing treatments. Beyond 1mM, fruits showed the phyto-toxicity symptoms. Hence, 1 mM concentration of both SA and MeJA were chosen for further analysis of tomato fruits for lycopene, AsA and total phenol content. Water-treated fruits were served as control. Fruits were kept separately at 20°C. Various traits were measured at 5, 10, 15 and 20 days after treatments. Each treatment contained three replicates of 10 fruit and the entire experiment was performed thrice.

Lycopene content

A method of Fish *et al.*, was adopted for evaluating the content of lycopene in tomato fruits [19]. A 0.5 g of homogenates of chopped tomato fruits was mixed with 0.05 % (5 ml; w/v) solution of known antioxidant BHT in acetone, consisting ethanol (5 ml) and hexane (10 ml). The mixture solution was kept on ice with stirring for 15 min following addtion of deionized water (3 ml) to each vial, and the samples were kept on shaking for next 5 min on ice. Further, the samples were kept at room temperature for 5 min for phase separation. Hexane fraction (upper layer phase) was taken and spectrophotometric measurements were made at 503 nm along with a blank control containing only hexane. The contents of lycopene were measured using the following formula:

$$\text{Lycopene content (mg/kg)} = \frac{A_{503} - 0.0007 \text{ X } 30.3}{g \text{ tissue}}$$

A_{503} = Absorbance of the sample at 503 nm

Total phenol content

A method described by Mallick and Singh was adopted for measuring the total phenolic contents [20]. Ethanol (2.5 ml) was added to tomato samples (0.5 g) followed by centrifugation at 4°C for 10 min. Supernatant was collected and re-extracted with 80 % ethanol (2.5 ml) followed by centrifugation. The supernatants were pooled and subjected to dry followed by addtion of distilled water (3 ml) to the dried material. Further, Folin phenol reagent (0.5 ml) and 20 % sodium carbonate (2 ml) were added to the reaction material. Then, the reaction solution was set in a boiling water-bath for one minute. Spectrophotometric measurements were done at 650 nm. Total phenol content was expressed as mg tannic acid 100 g/ fresh wt of tomato.

Ascorbic acid (AsA) content

Ascorbic acid content was determined by using the dye method as given by Albrecht [21]. 0.5 g of tomato samples were extracted with 3 % metaphosphoric acid followed by shaking at 300 rpm for 10 min. The extract was centrifuged at 10,000 rpm for 5 min. Serially diluted samples of known antioxidant such as ascorbic acid were prepared to draw the standard curve using metaphosphoric acid (3 %). Further, 1 ml of sample extract or standard compound was added into 3 ml of 0.2 mM 2, 6-dichlorophenol-indophenol (DCPIP) followed by spectrophotometric measurement at 515 nm. Amount of ascorbic acid was expressed as mg per 100 g of fresh weight. The experiment was replicated with three independent assays.

Statistical analysis

The data values of randomized experiments were collected as the mean ± SD followed by statistical analysis using ANOVA with least significance difference (LSD) at P d" 0.05.

Results

Lycopene content

Lycopene content was found to be increased in all the treatments during 15 days and declined thereafter. Among the treatments, SA treated fruits showed the highest lycopene content (199.57%) followed by MeJA (176.37%) in comparison with control (Fig. 1).

Ascorbic acid content (AsA)

AsA content was highest at 15th day in SA treated fruits (231.99 %) followed by MeJA (210.62 %) in comparison to control fruits and thereafter their content decreased. AsA content of SA and MeJA treated fruits was almost similar to control fruits at 20 days (Fig. 2).

Total phenol content (TPC)

Phenols accumulation in tomato fruits dipped with SA and MeJA was significantly higher in comparison with control fruits. Some amount of phenol was also detected in control fruits. SA treated fruits showed higher level of phenol at 15 days, which was 139.41 % higher than control fruits (Fig. 3). In general, TPC was found to be maximum in SA treated fruits, followed by MeJA. A downward trend, however, appeared after maximum induction at 15 days.

Discussion

Tomatoes are a very good source of antioxidants, vitamins C, carotenoids (lycopene and β-carotene) and phenolic compounds [22,23]. The present study validates positive influence of chemical inducers (SA and MeJA) on fruit quality in terms of antioxidant and defense properties

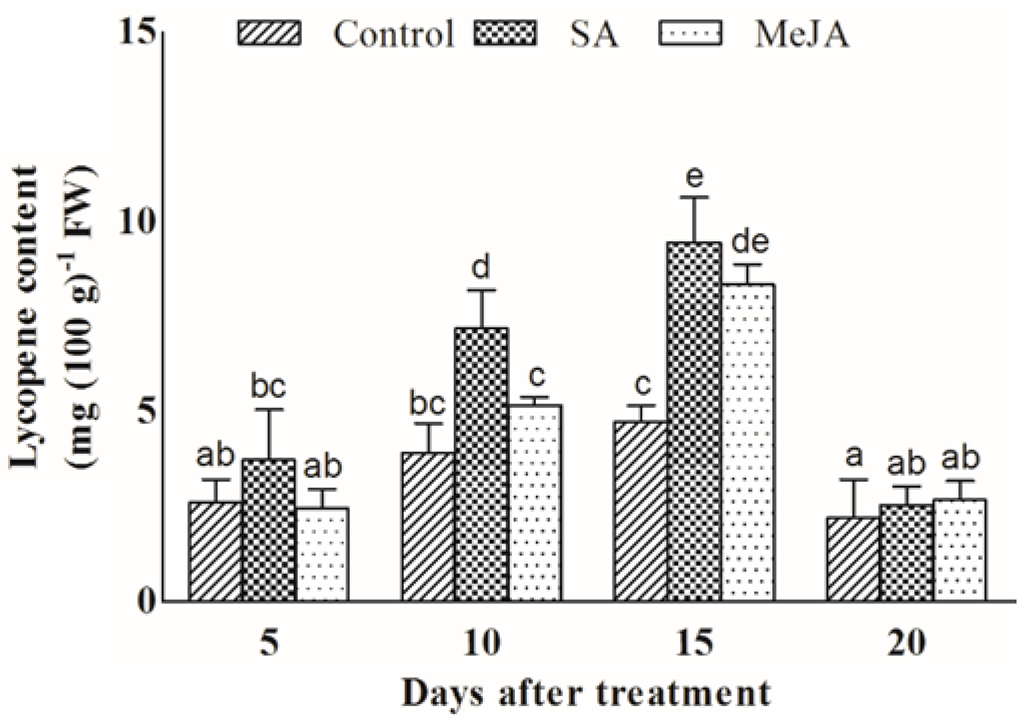

Fig. 1. Effect of SA and MeJA on lycopene content of tomato fruits. Different letters indicate significant differences among treatments within the results taken at the same time interval according to Duncan's multiple range test at $p \leq 0.05$

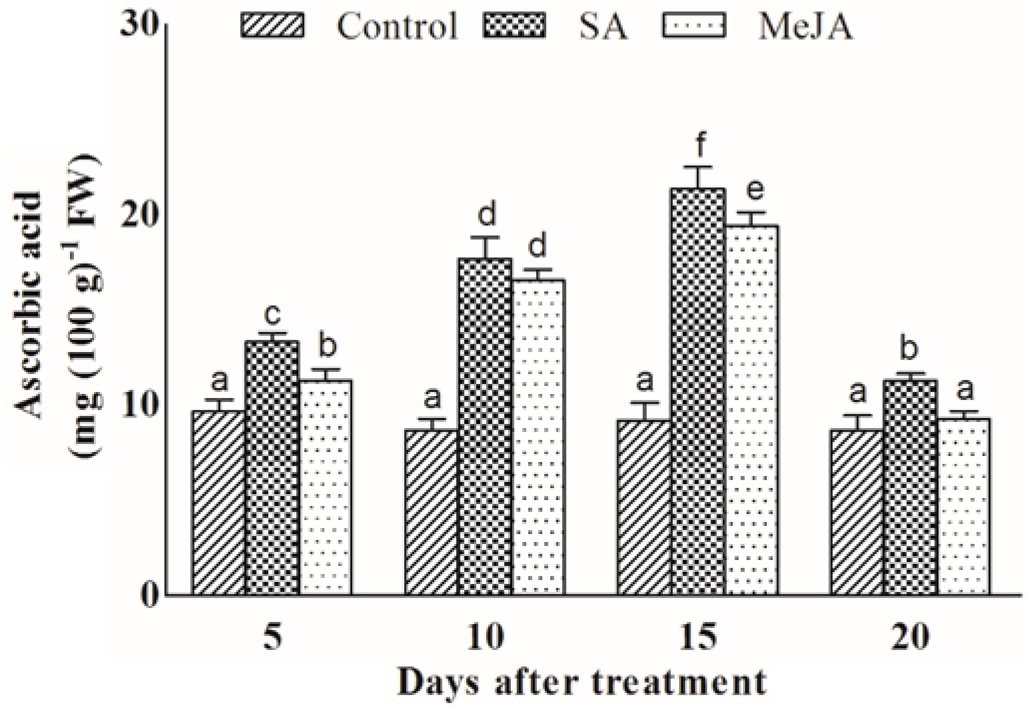

Fig. 2. Effect of SA and MeJA on ascorbic acid content of tomato fruits. Different letters indicate significant differences among treatments within the results taken at the same time interval according to Duncan's multiple range test at $p \leq 0.05$

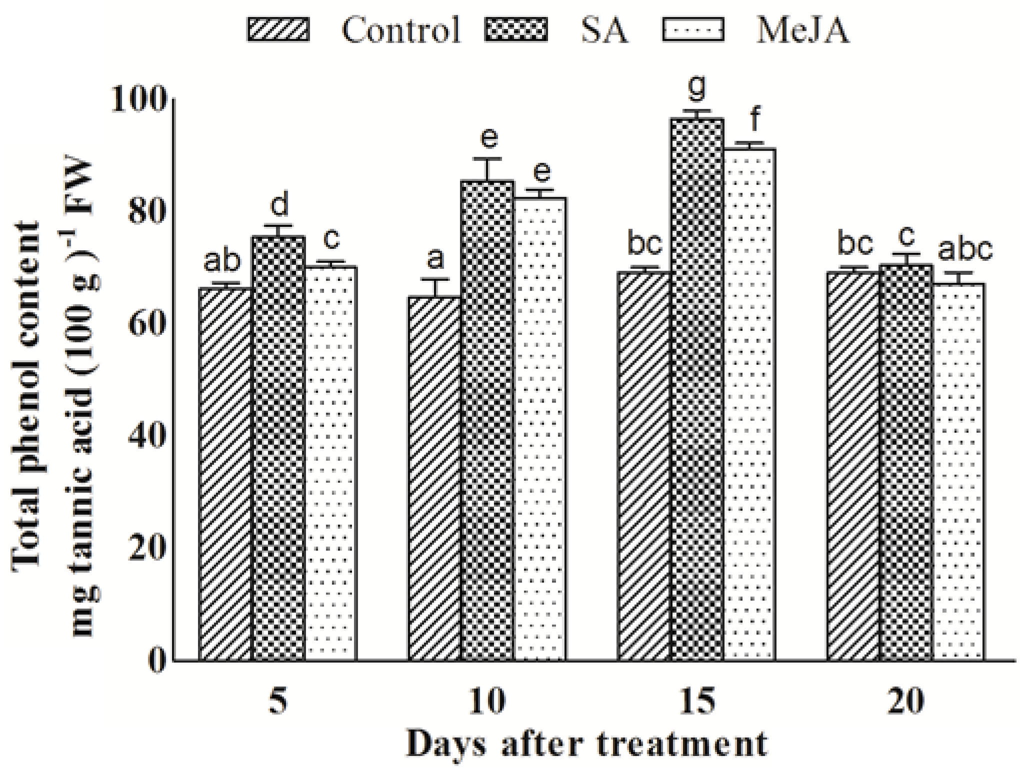

Fig. 3. Effect of SA and MeJA on total phenol content of tomato fruits. Different letters indicate significant differences among treatments within the results taken at the same time interval according to Duncan's multiple range test at $p \leq 0.05$

of tomato. Previously SA as an exogenous applicant has been found to maintain the quality characteristics of strawberry fruits [24]. In our study, maximum lycopene content was found in SA treated fruits. SA was reported to activate the synthesis of carotenoids and xanthophylls [25]. Similarly the effect of JA on the biochemical profile of pre-treated plant samples was analyzed by Liu *et al*.[26] and reported that lycopene synthesis was reduced by about 40 % in JA-deficient mutants, *spr2* and *def1* during fruit ripening, suggesting a positive impact of JA in the formation of lycopene conent.

Previously known facts are that higher contents of AsA represent ripening stage of fruits whereas senescent of fruits belongs to low AsA contents [27]. In our results, It was found that AsA content was highest in SA treated fruits followed by MeJA during 15 days in comparison to untreated samples and thereafter their content decreased. Earlier reports have confirmed efficacy of SA on maintaining AsA contents in orange fruits which has supported with higher antioxidant present in the orange fruits [28]. In another study, exogenous application of SA has been found to prevent AsA content loss during post-harvest storage of various fruits [29]. Kazemi *et al*.[30] reported quality maintaining ability of kiwi fruits by the joint treatment of SA and Ca during post-harvest storage. Also as reported previously, SA treatment had significant impact on enhancing AsA contents in tomatoes when compared with the control sets, suggesting enormous efficacy of SA to maintain quality of fruits [17].

Phenolic contents present in fruits and vegetables represent a vast variety of significance due to their various nutritional, sensory (color and flavor) and pharmacological attributes [31]. In this study, it was observed that tomato fruits treated with SA have increased phenol content in tomatoes in comparison to control fruits which might be due to enhancement of different enzymes and increased electronic transfer-based antioxidant that have increased total phenol content in SA treated fruits during storage. Yao and Tian [15] also confirmed that application of SA was able to delay phenolic component biosynthesis in ripening tomatoes. It has been observed that application of SA results in the increase activity of phenylalanine ammonia lyase during ripping, resulting in the enhanced biosynthesis of phenolic compounds. Also peach and tomato fruits treated with SA have been found to exhibit higher phenolic contents as compared to control group fruits [32,33].

Conclusion

In conclusion, it can be said that application of chemical inducers, especially SA prolonged the ripening with better quality traits of harvested tomatoes by increasing the synthesis of lycopene, ascorbic acid and total phenol content as compared to control fruits.

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