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Exogenous Pre-Harvest Treatment with Methyl Jasmonate and Chitosan Elicits Lycopene Biosynthesis in Tomato Plants

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Abstract: In this study, exogenous pre-harvest treatment of tomato plants with MeJA (Methyl Jasmonate) and chitosan was used to elicit lycopene biosynthesis and stimulate plant growth. Lycopene is a bright red carotene and carotenoid pigment found in tomatoes, carrots and other red fruits and vegetables. It is a powerful antioxidant that protects cells from damage and blocks cancerous cell growth. Many studies suggest eating lycopene-rich foods may be directly linked to reducing risk of several cancers, heart disease and stroke, hence there is a need to enhance its levels in tomatoes. Tomato seeds were soaked in MeJA and chitosan solutions of varying concentrations and then planted. The resulting plants were irrigated with the solutions used to soak the seeds, accordingly. The heights of the plants were recorded on a tri-weekly basis and HPLC (High Performance Liquid Chromatography) was used to analyze the effects of the MeJA and chitosan solutions on lycopene yields in the tomato fruits. Analysis of chromatograms obtained from tomato samples in the MeJA and chitosan-treated groups showed enhanced levels of lycopene. Plant growth was not significantly affected by treatment of MeJA. Chitosan, however, noticeably increased plant growth over 7 weeks.

Key words: Lycopene, jasmonates, chitosan, elicitation, tomatoes.

1. Introduction

Lycopene is a bright red carotene and carotenoid pigment found in tomatoes, carrots and other red fruits and vegetables [1]. In humans, it is a powerful antioxidant that targets free radicals, protects cells from oxidative damage and blocks cancerous cell growth [1]. Studies suggest lycopene helps prevent prostate, stomach and lung cancers as well as reduce the spread of pancreatic, colon, rectal, cervix and breast cancers [2]. Adequate dietary intake of lycopene-rich foods like tomatoes has also been linked with a decreased risk of cardiovascular disease and strokes [3].

Jasmonates (phytohormones including jasmonic acid and its related compounds) are an extensively-studied group of plant growth regulators. These linolenic acid-derived cyclopentanone-based hormones are known to play a vital role in fruit ripening, root growth and plant resistance to insects and pathogens [4]. In particular, they have been shown to play a vital role in regulating the biosynthesis of several secondary metabolites [5]. A jasmonate in particular, MeJA (Methyl Jasmonate) has been shown to stimulate the biosynthesis of several secondary metabolites such as stilbene in leaves and berries of grapevine plants [6]; anthocyanin in soybean seedling [7] and apples [8]; ß-carotene in tomatoes [9] and Caffeoylputrescine in tomato leaves [5]. Among the group of other known secondary metabolites stimulated by Jasmonates are quinones, alkaloids, terpenoids, polyamines, phenylpropanoids, antioxidants and glucosinolates [10].

MeJA has been known to play an important role in the biosynthesis of lycopene and other carotenoids in plants [11]. In one study, harvested tomato fruits treated with MeJA vapour showed increased lycopene accumulation [12]. There is no information on the effects of exogenous pre-harvest accumulation of lycopene. If it can be proven that pre-harvest treatment

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of plants with MeJA stimulates lycopene accumulation, a case could be made for MeJA being used as a fruit enrichment tool and MeJA-treated tomatoes, a potential therapeutic agent for various cancers.

Chitosan is a linear polysaccharide obtained by treating shrimp and other crustacean shells with sodium hydroxide [13]. It is a versatile compound that can be used as biopesticide [14], for food preservation [15] and in bandages to stop bleeding and reduce blood loss [16]. More importantly, studies have shown chitosan to be a very effective elicitor, playing a vital role in secondary metabolism and plant growth [17]. It was particularly effective in eliciting the accumulation of several plant polyphenols with antioxidant properties like stilbenes and flavonoids, when applied to plant cell cultures *in vitro* [18]. Its effect on several other polyphenols like Lycopene and ß-carotene has yet to be investigated.

Chitosan-treatment cell culture studies have also shown promising results in increasing the accumulation of polyphenols similar in structure and biosynthesis to lycopene [18]. There is little information, however, on the effect of exogenous chitosan treatment on plant growth and accumulation of lycopene.

Tomatoes are an excellent model for the study because one of their characteristics is a massive accumulation of carotenoids, notably lycopene.

2. Materials and Methods

2.1 Plant Materials and Growth Conditions

Cherry tomato (Meyler©) seeds were used in this study. Three MeJA solutions (0.1 µM, 0.5 µM and 1.0 µM) were prepared by dissolving 0.02 µL, 0.1 µL and 0.2 µL of 95% Sigma Aldrich brand MeJA. Three chitosan solutions were also prepared (0.1%, 0.5% and 1.0%, m/v) by dissolving 1 g, 5 g and 10 g of chitosan (Sigma-Aldrich) in 1 L of 0.25% (v/v) acetic acid solution. The tomato seeds were divided into eight groups of about a dozen each. Four groups were to be treated with one of the three MeJA solutions or distilled water (negative control), and another four groups to be treated with one of the three chitosan solutions or 0.25% acetic acid solution (negative control). The seeds were placed in petri-dishes and soaked for an hour with their respective solutions. The seeds were then removed and allowed to air-dry on filter paper for thirty minutes. 24 pots were filled with Miracle-Gro© potting mix and divided into eight groups of three, representative of the three MeJA groups, three chitosan groups, the MeJA control group and the chitosan control group. Into each corresponding pot, six seeds were sown.

The seedlings were grown in a greenhouse with temperatures ranging between 22-28 ºC and a 16 h photoperiod. Watering was done on Tuesdays and Thursdays with 500 mL of the corresponding solution for each group. Seedling height, leaf size and color were recorded in two week intervals. After harvest, fruits were quickly sealed in plastic zipper bags and frozen in the dark at -29.1 ºF to prevent metabolite degradation [19].

2.2 Metabolite Extraction

To analyze the lycopene content of the tomato fruits, homogenized with 20 mL of Chloroform and 10 mL of water in a Genemate© 35 mL centrifuge tube using a Benchmark© D1000 homogenizer, then magnetically stirred for 5 minutes. The extracts were centrifuged at 3,000 rpm and 4 ºC for 15 minutes. Three distinct layers were visible: an aqueous layer, a second layer consisting of fruit debris and a third metabolite layer. 10 mL of the metabolite layer was removed and a pinch of $NaHCO_3$ was added to remove any moisture present. A Labnet© VX100 vortex mixer was used to mix the metabolite solution which was then evaporated to dryness using nitrogen gas in a Techne© sample concentrator. The resulting metabolite powder was dissolved in 10 mL of chloroform and prepared for HPLC analysis.

2.3 HPLC Analysis of Lycopene Content

Analysis was performed using a Shimadzu LC-20A

Series HPLC system consisting of a Shimadzu DGU-20AR pump set at pressure 500 psi, a Shimadzu LC-20AP solvent delivery unit set at a flow rate of 1.5 mL·min^{-1}, and an SPD-20A detector with a D2 lamp and a built-in Hg for wavelength accuracy check. A Hypersil C18 column (5 µm particle size, 4.6 × 250 mm; Elite Analytical Instruments Co., Ltd., Dalian, China) was used with a mobile phase of methanol/acetonitrile (80/20 v/v). The total retention time was 15 min. A 20 µL sample was injected into the column manually. Absorbance was detected at 450 nm and 371 nm. Standards were prepared and used to identify and quantify the corresponding carotenoids.

3. Results

HPLC was used to analyse the carotenoid content in the extracts. Lycopene was identified by comparing the peaks obtained to those from chromatograms of standard lycopene solutions. To determine whether lycopene levels were in fact increased, peak area was used to compute total lycopene concentration in mg/mL using Shimadzu Labsolutions Suite©. The following figures (1C, 2, 3 and 4) and Table 1 show total lycopene content obtained from the chromatograms after analysis of samples from every group.

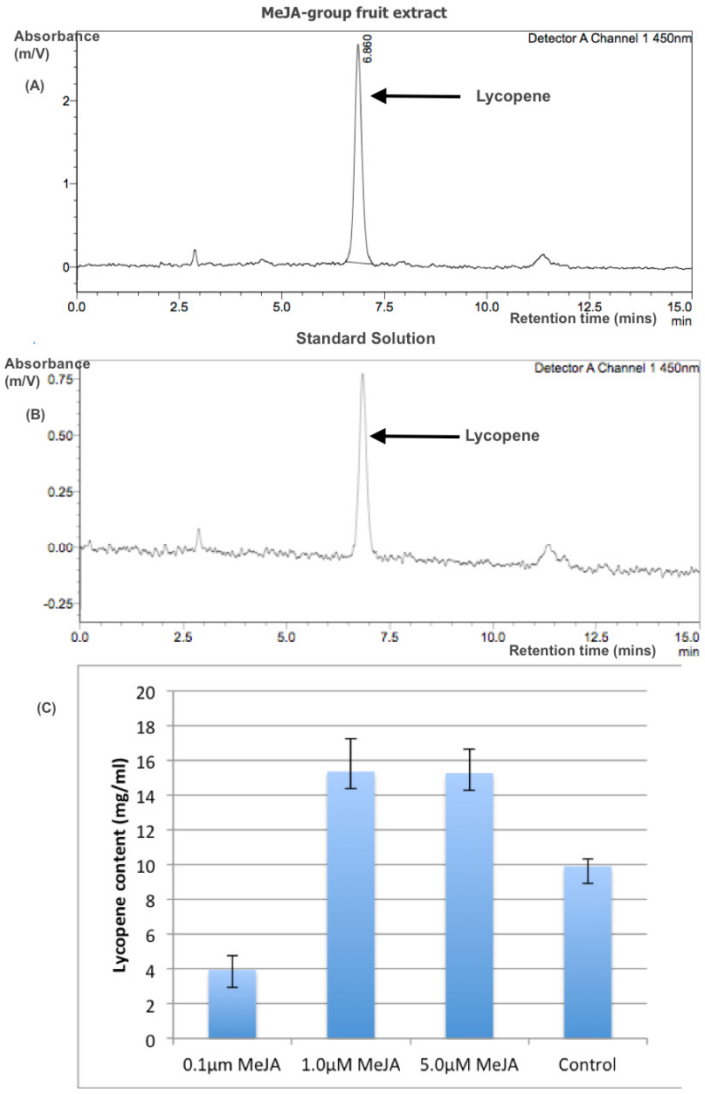

Fig. 1 (A) and (B) Chromatographic separation of lycopene extracted from tomato fruits relative to standard lycopene solution. (C) Effects of MeJA on lycopene content in tomato fruits.

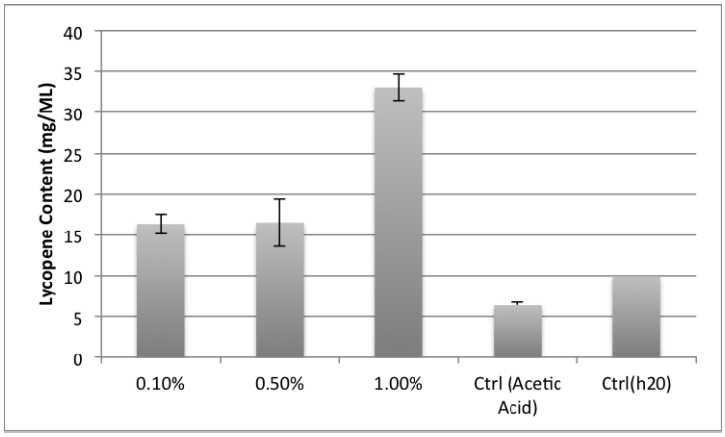

Fig. 2a Lycopene levels in tomato extracts from the chitosan, acetic acid control and water control groups.

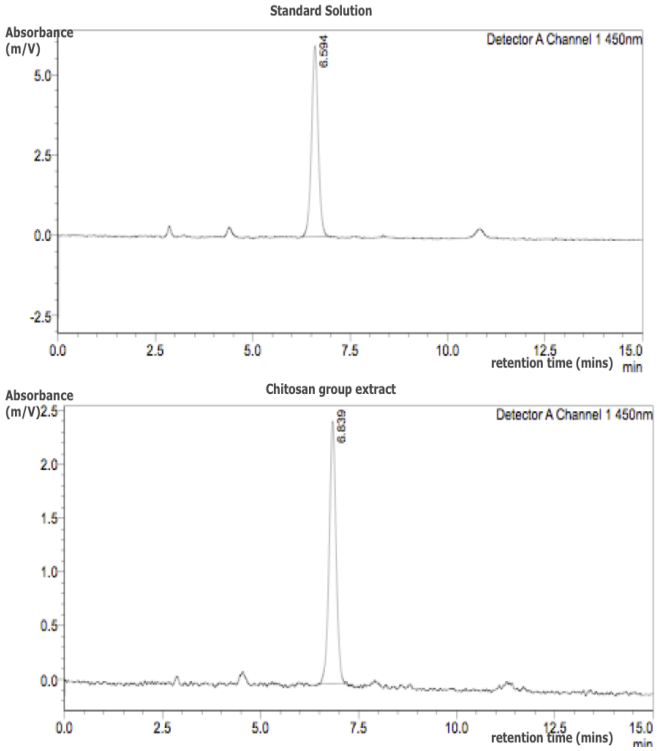

Fig. 2b Chromatographic separation of lycopene obtained from chitosan treated tomatoes relative to standard lycopene solution.

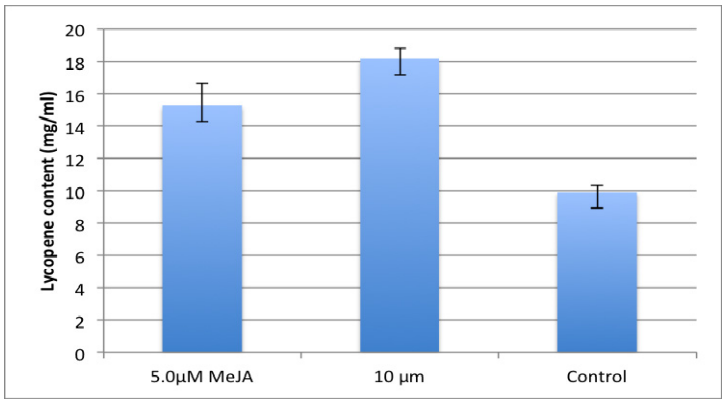

Fig. 3 Lycopene levels in tomatoes from 5.0 µM, 10 µM and control groups.

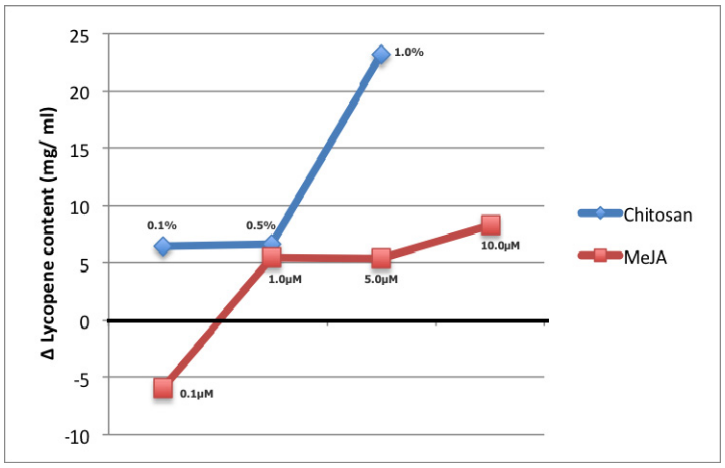

Fig. 4 Change in lycopene levels as a result of treatment with chitosan and MeJA. Difference between lycopene level averages in samples vs. lycopene averages in water control.

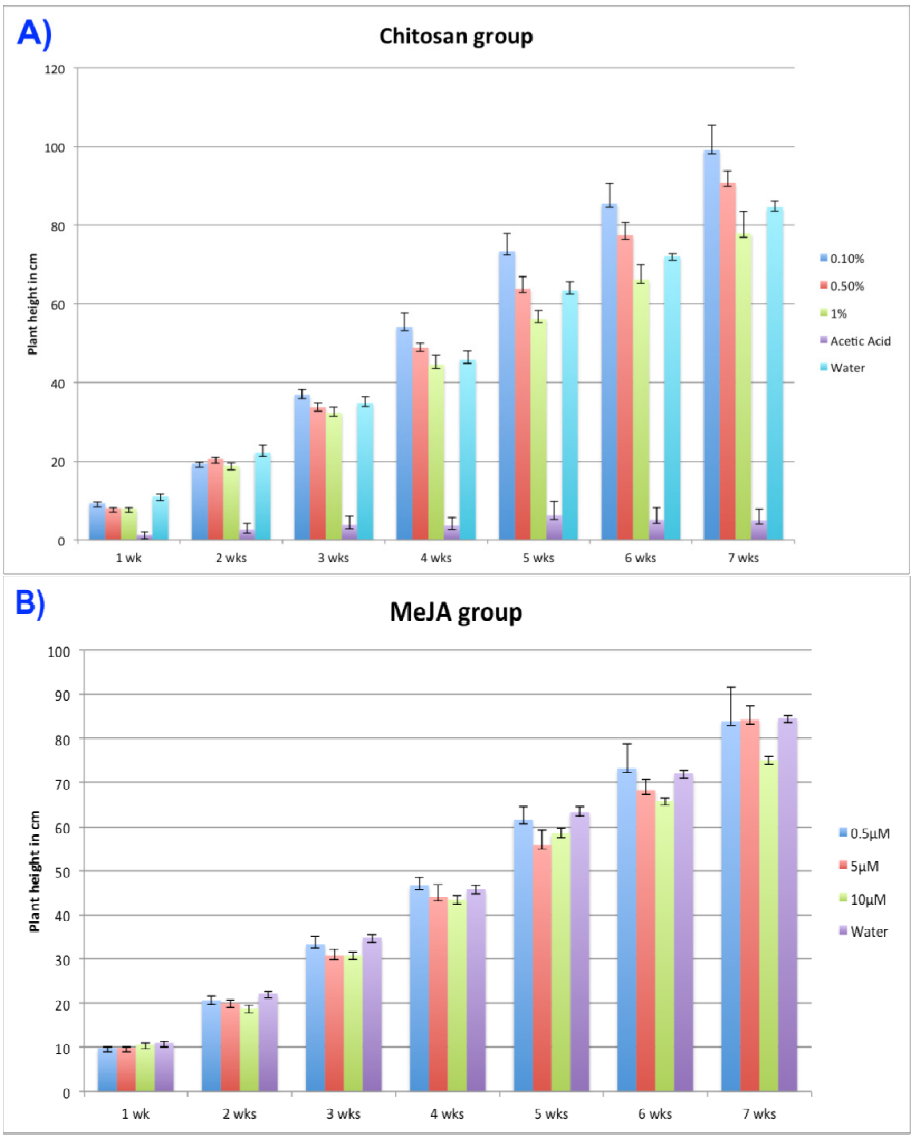

Fig. 5 Growth data showing plant height over 7 weeks for both MeJA and water control groups (B) as well as chitosan, AA and water control groups (A).

Table 1 Effect of Methyl Jasmonate and chitosan on lycopene levels in tomato fruits. Lycopene content data expressed in mg/mL.

Treatment	Trial 1	Trial 2	Trial 3	Average ± standard error
0.1% Chitosan	18.69	14.7	15.64	16.3 ± 1.2
0.5% Chitosan	12.88	14.38	22.27	16.51 ± 2.9
1.0% Chitosan	34.99	31.09	--	33.04 ± 1.6
Control (AA)	6.37	7.01	5.7	6.36 ± 0.4
0.1 μm MeJA	2.29	4.72	4.81	3.94 ± 0.8
1.0 μm MeJA	11.84	18.26	16.0	15.4 ± 1.9
5.0 μm MeJA	16.57	12.49	16.78	15.28 ± 1.4
10.0 μm MeJA	18.37	19.22	16.9	18.16 ± 0.7
Control (water)	9.92	9.21	10.59	9.91 ± 0.4

Generally, lycopene levels were significantly higher in extracts obtained from plants treated with Methyl Jasmonate and Chitosan (Figs. 1 and 2) than those in the control groups. In plants treated with 0.1 μM MeJA, however, lycopene levels were lower (Fig. 1). On average, 1.0 μM had the best lycopene yields in the MeJA group (Fig. 1). These yields were only marginally higher than those in the 5.0 μM group, with both groups having significantly more lycopene than the water control group (Fig. 1).

In the chitosan group (Fig. 2a), lycopene levels were significantly highest in the 1.0% group than in any other group. Lycopene levels were also much lower in the acetic acid control group than in both water control and chitosan control groups.

To further test the effect of MeJA on lycopene levels, another concentration (10 μM) of MeJA was used to treat plants grown under identical conditions to those in the first study. The results (Fig. 3) showed MeJA affected lycopene levels in a dose-dependent manner, with lycopene levels in the 10 μM group ~85% higher than those in the control, but only 19% higher than those in the 5.0 μM and 1.0 μM group.

Chitosan was much more efficient in eliciting lycopene accumulation than MeJA (Fig. 4). Even in low concentrations, lycopene levels were increased tremendously compared to both MeJA and control groups.

Plant growth appeared to be largely unaffected by MeJA treatment (Fig. 5B). Chitosan treatment appeared to boost plant growth with the plants standing noticeably taller than both the water and AA control plants. Acetic Acid treatment impeded plant growth significantly (Fig. 5A).

4. Discussion

Jasmonates are phytohormones that regulate the synthesis of many secondary metabolites in tomatoes [5]. They play a notable role in the biosynthesis of carotenoids [11]. Early studies suggested that exogenous MeJA treatment in low doses reduced lycopene and beta-carotene levels in tomato fruits [9]. Authors used MeJA of varying concentrations revealed, MeJA affected lycopene yields in a dose-dependent manner. In low concentrations (0.1 μM and 0.5 μM), it hindered lycopene accumulation. This is consistent with results obtained in previous studies where exogenous application of a 0.5% solution of MeJA in lanolin paste reduced lycopene accumulation [9]. In higher concentrations (1 μM, 5 μM and 10 μM), however, lycopene accumulation was drastically increased, with as much as 63% more lycopene in the 10 μM group than in the controls (Fig. 2a). Jasmonates play an important regulatory role in the biosynthetic pathway for lycopene [5]. It is possible that in ripening tomatoes, small quantities of MeJA inhibit the conversion of phytoene to lycopene, which reduces accumulation of lycopene [20]. In high concentrations, this effect appears to be reversed.

In spite of the concentration parameters, the differences in response could be due to the method by which the MeJA was administered to the plants. In

previous studies, MeJA was administered in lanolin paste [9], which prolonged the treatment time. In other MeJA-metabolite studies, MeJA is administered as a vapour, while in this one, the plants were irrigated with MeJA solutions, which reduced overall treatment time.

Chitosan is an effective plant elicitor that has been shown to increase accumulation of health-beneficial secondary metabolites, notably polyphenols such as stilbenes, anthocyanin and other flavonoids [21]. In fact, in many comparative studies with other elicitors like salicylic acid, Jasmonates and antibiotics ampicillin and rifampicin, chitosan consistently yielded better results than the others [18]. Many metabolites, mostly polyphenols, in plants are produced through the Phenylpropanoid pathway. Chitosan up-regulates specific target branches of this pathway, promoting the accumulation of several polyphenols like lycopene in a dose-dependent manner [18]. This explains why lycopene levels were significantly higher in the Chitosan groups than in both controls. It also explains the impressive levels of lycopene in the 1.0% chitosan group.

Although plant growth was largely unaffected by treatment with MeJA, there is evidence to suggest jasmonates play a positive role in plant growth [22]. Plant cell culture studies have also shown chitosan to be effective in stimulating plant growth [17]. The plants in the acetic acid group appeared to be stunted. Acetic acid's toxic effect on plants is been well documented. In adequate doses, acetic acid impedes plant growth and could function as an herbicide [23]. This could be because acetic acid causes degradation of membrane protein in plants [24].

In conclusion, exogenous pre-harvest treatment of tomato plants with MeJA and chitosan has a dose-dependent, positively regulatory effect on lycopene accumulation. In low concentrations, lycopene accumulation is reduced, but in high concentrations, it is increased drastically. In lower concentrations, chitosan increased lycopene accumulation. This effect was more pronounced in higher concentrations. The findings have improved our understanding of lycopene biosynthesis and shown that jasmonates and chitosan play a key role in secondary metabolite biosynthesis.

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