Sains Malaysiana 51(11)(2022): 3635-3646
http://doi.org/10.17576/jsm-2022-5111-10

Tomato Post-Harvest Fruit Ripening in Pakistan: Effect of Methylcyclopropene (1-MCP) in Inhibiting Ripening Gene Expression

(Pemasakan Buah Tomato Selepas Dituai di Pakistan: Kesan Metilsiklopropena (1-MCP) dalam Merencat Pengekspresan Gen Pemasakan)

Aleena Ramzan, Rida Javed, Alveena Zulfiqar & Samina N Shakeel*

Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan

Received: 24 September 2021/Accepted: 31 July 2022

ABSTRACT

Short shelf life of tomato fruits, with the unavailability of modernized handling/storage and transport practices causes ~30-40 % of yield loss which leads to great financial burden on the import of Pakistani tomatoes. There is an urgent need to adapt an easy and cost effective applicable technology for increasing the shelf life of fully ripened tomato fruits by inhibiting ethylene perception that leads to ripening and decay. This study aims to evaluate the effectiveness of ethylene perception inhibitor 1-methylcyclopropene (1-MCP) on ripening of tomatoes. Physiological and molecular parameters were recorded to analyse the inhibitory effect of 1-MCP. Green tomatoes were harvested and treated with 1-MCP for 24 h and 8 days. Results of the physiological data based on colour, weight, and firmness of the fruits showed a significant delay in the onset of ripening after 1-MCP treatment. 1-MCP is known to block ethylene receptors, therefore, we used different ethylene and ripening related genes as markers to prove that 1-MCP potentially delayed tomato fruit ripening by inhibiting the ethylene pathway that in turn downregulated the ripening signalling pathway genes. Therefore, this study provides evidence that 1-MCP can effectively be used as an alternative to reduce the post-harvest tomato yield losses in Pakistan with slight optimization on the method to suit the different varieties of local tomatoes.

Keywords: Delayed ripening; ethylene; 1-MCP; ripening gene

ABSTRAK

Jangka hayat buah tomato yang pendek serta ketiadaan amalan pengendalian/penyimpanan dan pengangkutan moden telah menyebabkan kehilangan ~30-40% hasil tomato yang turut mengakibatkan bebanan kewangan yang besar ke atas import tomato Pakistan. Terdapat keperluan mendesak terhadap penggunaan teknologi yang mudah dan berkesan dari segi kos untuk meningkatkan jangka hayat buah tomato masak dengan menghalang persepsi etilena yang mengawal pemasakan dan pereputan. Kajian ini bertujuan untuk menilai keberkesanan perencat persepsi etilena 1-metilsiklopropena (1-MCP) terhadap pemasakan tomato. Parameter fisiologi dan molekul direkodkan untuk menganalisis kesan perencatan 1-MCP. Tomato hijau dituai dan dirawat dengan 1-MCP selama 24 jam dan 8 hari. Hasil data fisiologi berdasarkan warna, berat dan kepejalan buah menunjukkan kelewatan yang ketara dalam permulaan pemasakan selepas rawatan 1-MCP. 1-MCP diketahui menyekat reseptor etilena, oleh itu kami menggunakan gen berkait etilena dan pemasakan sebagai penanda untuk membuktikan bahawa 1-MCP berpotensi untuk menangguhkan pemasakan buah tomato dengan merencatkan tapak jalan etilena yang seterusnya mengawal atur menurun gen untuk tapak jalan isyarat pemasakan. Oleh itu, kajian ini membuktikan bahawa 1-MCP boleh digunakan secara berkesan sebagai alternatif untuk mengurangkan kehilangan hasil tomato lepas tuai di Pakistan dengan sedikit pengoptimuman pada kaedah untuk disesuaikan dengan varieti tomato tempatan yang berbeza.

Kata kunci: Etilena; gen peranuman; penangguhan pemasakan; 1-MCP

Introduction

Tomato (*Lycopersicum esculentum*) is known to be the vital fruit used worldwide. Tomato belongs to the *Solanaceae* family (Khokhar 2013). Tomatoes are part of balanced and healthy diet. Tomatoes can be consumed raw in salads and several dishes like soups, sauces, cooked vegetables, meat, and fish to make gravy purées, juices, and ketchup. Tomatoes are rich sources of dietary fibers, vitamins, minerals, flavonoids, carotenoids (β-carotene, xanthophyll

and lycopene), phytochemicals and many phenolics (Frusciante et al. 2007), which help in preventing many chronic diseases including cardiovascular disease (CVD) and cancers. Tomatoes are necessary for daily diet due to their anti-mutagenic, anti-cancerous, and antioxidant activities due to carotenoids and phytochemicals (Rao & Agarwal 1998). Ripened tomato fruit usually has a very short shelf life and can be stored for up to 7-10 days only after picking. Pakistani farmers lack proper training and technological facilities to enhance the shelf life that in turn results in loss of tomato yield due to spoilage by over ripening (Khokhar 2013). To prolong the shelf life of tomatoes, several handling, storage, and transport practices are being used (Mursalat et al. 2013). Pakistan has to import tomatoes from Afghanistan, Iran, and India because the locally produced tomatoes are not enough due to lack of storage facilities. Tomatoes from Baluchistan do not fully ripen due to cold weather, and tomatoes from Sindh cannot be usually consumed due to unexpected delays/damages during transportation to other parts of the country during warm weather without temperature-controlled containers. The federal government thus decided to import tomatoes from other countries to overcome the shortage (Rizvi 2017). Although post-harvest experts have controlled this issue and have increased the storage time of fruits and vegetables by controlling humidity and temperature conditions to keep the fruits fresh in a controlled atmosphere in advanced countries (Huber, Jeong & Ritenour 2003), but Pakistan lacks the advanced transportation facilities that results in loss of tomato yield due to spoilage and over ripening.

Scientifically, fruit ripening is considered a process during which the fruit undergoes a change at biochemical and physiological levels. These developmental changes are reflected by the fruit appearance, aroma, texture and flavor (Giovannoni 2004). Production of endogenous ethylene triggers the natural ripening of climacteric fruits like tomatoes. Ethylene activates and regulates many underlying ripening related changes that occur at the physiological and molecular level in such fruits (Moretti et al. 2002). The recent ethylene production model describes ethylene signaling as a linear transduction pathway. A signaling cascade is started when specific ethylene receptors (ETR1, ETR2, ERS1, ERS2, and EIN4) perceive the ethylene hormone, and the downstream molecule EIN2, which was inhibited by CTR1 in the absence of ethylene gets released. The release of this block recruits a transcriptional factor, EIN3/EIL1 to translocate to the nucleus. Transcription factors Ein3/Eil1 and ERFs in the nucleus induce the expression of ripening genes in response to ethylene. These signaling events are responsible for all phenotypic traits in tomatoes such as colour, texture, aroma, firmness, decay and taste (Chang et al. 2013). The latest identifications of ripening associated molecular switches and transcriptional regulators have produced new insights towards understanding the ripening control in fleshy fruits. These ripening events were found to link directly or in concert with the ethylene (Liu et al. 2015). Some of the important ripening related regulators have been characterized recently, which include colourless non-ripening genes, ripening-inhibitor and non- ripening mutants (Manning et al. 2006).

Several synthetic compounds are available to slow down fruit ripening by blocking the action of ethylene, which suppress or inhibit ethylene action. Diazocyclopentadiene and 2,5-norbonadiene have shown the ability to control both ripening and softening of apples (Blankenship & Sisler 1993). Other compounds which can be potentially used for this purpose are aminooxyacetic acid (AOA), aminoethoxyvinylglycine (AVG), and silver thiosulfate (STS) (Abeles, Morgan & Saltveit Jr. 2012). However, none of these compounds are commercially acceptable due to toxicity and manufacturing concerns (Sisler 2006). In many advanced countries, 1-methylcyclopropene (1-MCP) has been used to increase postharvest life of many fruits (Biswas et al. 2014). 1-MCP has competitive affinity to ethylene receptors and blocks ethylene perception by preventing ethylene binding (Sisler & Serek 1997). MCP utilization in Apple postharvest technology is considered as a break-through for maintaining quality for consumers (Huber, Jeong & Ritenour 2003). 1-MCP maintains apple taste and firmness (crunchiness), sugar content (sweetness), and titratable acidity (tartness). 1-MCP is usually applied as a gas for a short time (2-24 h) at very low concentrations and being nontoxic, does not have any toxic effect on the fruit consumer (Huber, Jeong & Ritenour 2003). 1-MCP affinity for the receptors is almost ten times higher than that of ethylene, therefore it is considered as highly effective even at lower concentrations (Blankenship & Dole 2003).

Ripening process in fruits creates a significant difference in their morphological and developmental stages. Ripening is associated with increased ethylene levels in fruits. Transcription factors also regulate the ripening process in fruits. Ripening inhibitor (RIN), MADS box gene and CNR (Colourless non ripening), SPB box gene regulate the ripening process with ethylene in fruits (Gamrasni et al. 2020). In climacteric fruits,

ripening is initiated by the hormone ethylene. The ripening processes that include change in colour, aroma and firmness is also controlled by other ripening agents such as *RIN, CNR, NOR,* and *PSY* with ethylene (Li et al. 2020). These transcription factors together with ethylene control the expression of downstream ripening related genes (Wang et al. 2020). Phytoene synthase 1 (*PSY 1*) is the activating gene of the lycopene biosynthetic pathway. Lycopene is the enzyme that brings the bright red colour to ripened fresh tomato fruit. It converts geranylgeranyldiphosphate (GGPP) to phytoene which is then converted to lycopene and the red colour of fruit is produced (Giorio, Stigliani & D'Ambrosio 2008). The change in colour during the developmental stages of tomatoes is controlled by the chlorophyll degradation and a high content of carotenoids, one of which is lycopene. Enzyme responsible for the synthesis of carotenoids in tomatoes are phytoene synthase 1 (Psy1) (Fraser et al. 2007).

In the present study we investigated the effect of 1-MCP on tomato fruit ripening. We measured physiological and molecular parameters to identify exposure time required for 1-MCP to delay the ripening process. We also investigated the effect of 1-MCP to enhance shelf life of tomato. Therefore, this study provides strong evidence that 1-MCP is recommended as a cost effective, non-toxic and cheaper technological alternative that could be utilized to delay ripening of tomato fruits in Pakistan to reduce the postharvest losses.

Materials and Methods

Experimental design

Freshly harvested, non-damaged, green round-shaped tomato fruits (60-155 g) were used for further experimental analysis. Tomatoes were kept in three biological replicates in a closed container with a rubber septum for 1-MCP treatment for 24 h and 8 days. Control tomatoes were also kept in three biological replicates in a closed container for 24 h and 8 days. 1-MCP containing 3.3% active ingredient was obtained from Qing Dao Lu Nuo BioTechnology Co. Ltd., China. Ten uL/L of 1-MCP was applied through the rubber septum in air tight closed container and control fruits were kept at similar conditions without 1-MCP exposure at 25 °C.

Physiological parameters

For both 1-MCP treatments (24 h and 8 days), we analyzed physiological parameters including weight loss, firmness, and colour change during the storage period after 1-MCP treatment and control. Data were recorded on a daily basis for 16 days of storage at 25 °C as given below. Physiological parameters such as colour, weight, and firmness were checked in comparison with control fruits. Twenty experts of age group 24-40 years made the critical judgement on colour change of tomatoes. Firmness was checked by the finger feel firmness method (Batu 2004). Tomatoes were stored at 25 °C temperature after taking out from 1-MCP treatment containers.

RNA extraction and real time PCR

Tomato fruit tissues both fleshy parts and skin from treated and control samples were taken on the first day of storage for RNA isolation with TRIZOL method. For DNase treatment, DNA-free kit (Thermofisher Scientific) was used according to instructions. First strand cDNA was synthesized using the standard protocol of BIORAD iScriptc DNA synthesis kit for RT-qPCR according to manual. cDNA was prepared from 1 µg RNA and was diluted to five folds to be used for real-time PCR. Reaction was carried out with an initial incubation at 95 °C for 20 s, followed by 40 cycles of 95 °C for 10 s and 60 °C for 60 s with MYGO PRO Connect Real time PCR system.

The cycle threshold (Ct) $2^{-\Delta(\Delta Ct)}$ method was adopted for relative quantification of specific mRNA levels. For RT-qPCR, three technical replicates of each cDNA sample were analyzed with the BIORAD iTaq Universal SYBR Green supermix on MYGO-PRO Real-Time PCR system. SEs was calculated based on a minimum of three biological replicates. The gene-specific primers were designed using Integrated DNA technologies (IDT) Primer Quest SM (Table 1). *Phytoene Synthase 1* (*Psy1*) and three of its transcription factors, *Ripening inhibitor* (*RIN*), *Non-Ripening* (*NOR*), and *Colourless non-ripening* (*CNR*) genes were analyzed as markers for ripening. *ETR1* expression was analyzed to check the inhibitory effect of 1-MCP. *EF1-alpha* gene was used as a control gene.

Statistical analyses

Statistical analysis was accomplished by t-test with a Bonferroni correction and One-way ANOVA with post hoc Tukey HSD. Test Calculator was used for comparing multiple treatments (* $p \leq 0.05$; ** $p \leq 0.01$), statistics was applied to experimental samples and the wild-type controls in each case.

TABLE 1. Primers used for genes involved in the ethylene signal transduction and ripening

Gene Name	Forward (5'---- 3')	Reverse (5'----- 3')
ETR1	AGAGGGAGTTGTGAGCCAGA	GCTGCAGCCCTGACTATCTC
ACS	AAAAATAATGCGGGGCTTTT	TTGTTGCTTCTTTTCCATCG
RIN	AACATCATGGCATTGTGGTG	GTGTTGATGGTGCTGCATTT
CNR	GCCAAATCAAGCAATGATGA	TCGCAACCATACAGACCATT
NOR	AGAGAACGATGCATGGTTGT	ACTGGCTCAGGAAATTATGG
PSY1	ATCTTTGGTCTTGTACCGCAA	GGCAGTTTTTGTAGGAGGCA
EF1	GCTGTCGGTGTTGTCAAGAAT	CATCACACTGCACAGTTACT

Results

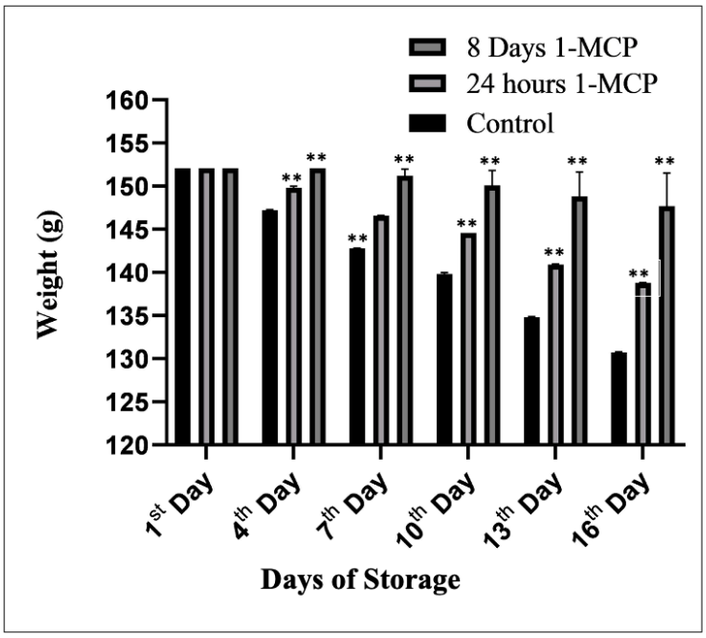
Effect of 1-MCP in delaying tomato ripening

Inhibitory effect of 1-MCP (methylcyclopropene) on tomato fruit ripening was analyzed on a daily basis for 16 days of storage. After 24 h and 8 days of treatment of 1-MCP, the tomatoes showed delay in the process of ripening compared to control.

Weight loss

Percentage weight loss was observed for all control and 1-MCP treated samples. Control samples showed a gradual decrease in weight. Weight of control tomatoes on average was 153 g on the 1st day of storage and reduced to 132 g on the 16th day of storage. Decrease in the weight shows that the fruit has lost its firmness and moves towards the decay of fruit. Tomatoes treated with 1-MCP for 24 h showed a decrease from 153 g on the first day of storage to 137 g on the 16th day of storage. Whereas tomatoes treated with 1-MCP for 8 days showed less decrease in weight compared to control and 24 h treated samples. Weight of 1-MCP treated samples for 8 days was reduced from 153 g on the first day of storage to 146 g on the 16th day of storage. Marked increase in weight loss was observed in control samples during their storage time due to natural ripening process. However, 1-MCP treated samples had less weight loss due to delay of ripening procedure by down regulating ethylene signaling. We observed that tomatoes treated with 1-MCP for 8 days had comparatively slower weight loss as compared with 24 h treated group (Figure 1).

Loss of firmness



Columns represent the weight of tomatoes in control, 24 h and 8-days of 1-MCP treated. Asterisks show significant difference between control, 1-DMT, and 8-DMT (** shows $p \leq 0.01$)

FIGURE 1. Weight loss data for 24-h and 8-days of 1-MCP treated tomatoes in comparison to control till 16 days of storage

We observed a gradual softening pattern in non-treated control tomatoes, whereas samples treated with 1-MCP for 24 h and 8 days exhibited reduction in firmness after 7 days of storage (Table 2).

TABLE 2. Loss of firmness in tomatoes after 1-MCP treatment

Days of storage	Control	24 h 1-MCP	8 Days 1-MCP
1st day	Hard	Hard	Hard
4th day	Hard	Hard	Hard
7th day	Soft	Hard	Hard
10th day	Soft	Soft	Soft
13th day	Soft	Soft	Soft
16th day	Rot	Soft	Soft

COLOUR CHANGE

One of the most prominent parameters of ripening is colour change. Initially, no colour change was observed in control tomatoes for the first four days. Control tomatoes turned orange on the 4th day and were completely red on the 10th day of storage (Figure 2). However, this colour change was significantly delayed in tomatoes treated with 1-MCP, 24 h treated samples with 1-MCP turned orange (pink stage) on the 7th day of storage, turned light red on the 10th day of storage and red on the 13th day of storage (Figure 2). Samples treated with 1-MCP for 8 days remained green till the 7th day of storage turned light red on 13th day of storage and complete red on 16th day of storage (Figure 3). Table 3 describes the colour change in tomatoes from 1st day to the 16th day of storage in Control, 1-MCP treated samples for 24 h and 8 days.

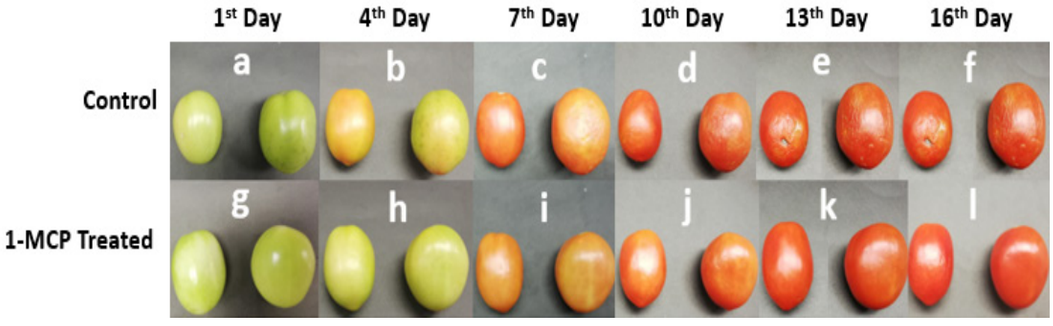


FIGURE 2. Colour change in tomatoes for 24 h 1-MCP treated tomatoes till 16th day of storage

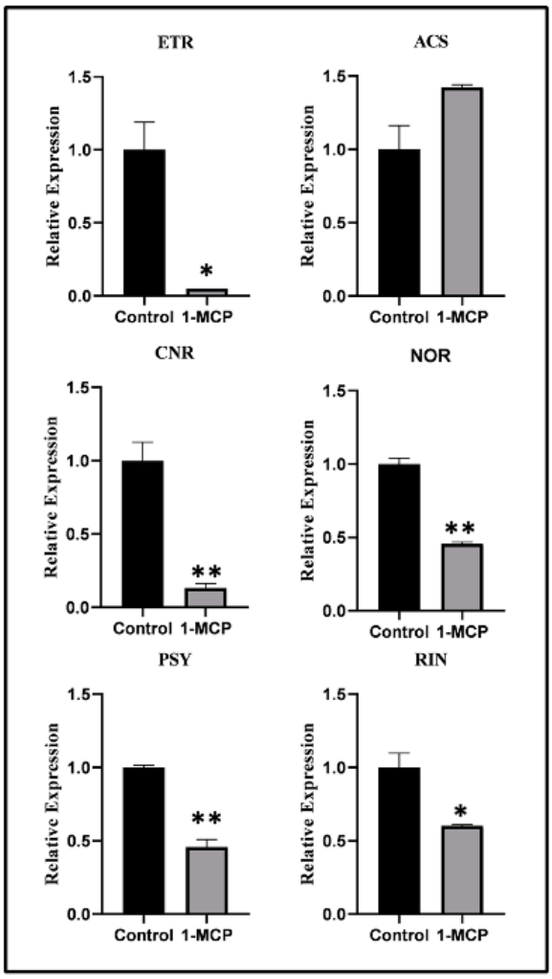
FIGURE 3. Colour change in tomatoes for 8 days 1-MCP treated tomatoes until 16th day of storage

TABLE 3. Colour change in tomatoes after 1-MCP treatment

Days of storage	Control	24 h 1-MCP	8 Days 1-MCP
1st day	Green	Green	Green
4th day	Orange	Green	Green
7th day	Light Red	Orange	Green
10th day	Red	Light Red	Green
13th day	Red	Red	Light Red
16th day	Red	Red	Red

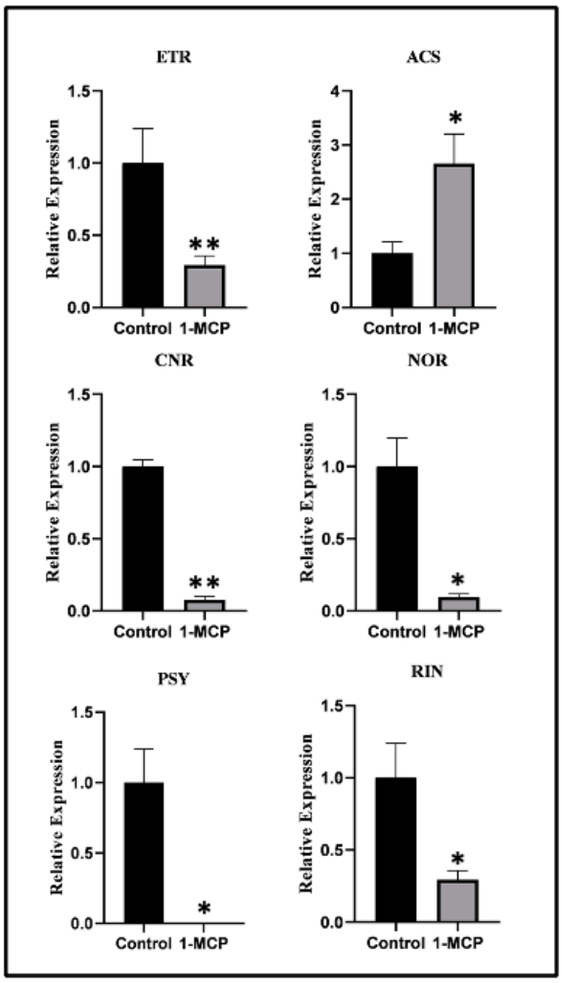
TRANSCRIPT LEVEL OF ETHYLENE INDUCIBLE RECEPTOR *ETR1* WERE DOWN-REGULATED IN RESPONSE TO 1-MCP

Expression pattern of ethylene receptor gene (*ETR1*) was analyzed with qRT-PCR to check the inhibitory action of 1-MCP on ethylene receptor. 1-MCP binds and blocks the ethylene binding with the receptors and thus down-regulates the expression of ethylene response genes (Street et al. 2015). Decrease in the expression of *ETR1* was observed in 24 h and 8 days treated tomatoes with 1-MCP as compared to control. Decrease in the expression of *ETR1* confirmed the inhibitory response of 1-MCP in the ripening of tomatoes even at endogenous levels of ethylene (Figures 4 & 5). Comparative analysis



Tomatoes were kept in the presence and absence of 1-MCP for 24 h and gene expression was examined by qRT-PCR. Expression was normalized to elongation factor-alpha control and is presented as relative to the untreated control. ETR1 and ACS are positive controls for ethylene-regulated gene expression. *RIN, NOR*, and *PSY1* are genes involved in the ripening of a fruit. The height of each column shows the relative mRNA abundance. Error bars indicate SE using three replicates. Asterisks show significant difference between control and 24 h 1-MCP (* shows $p \leq 0.05$, ** shows $p \leq 0.01$)

FIGURE 4. Expression of ethylene signaling and ripening genes in 24 h 1-MCP treated tomatoes



Tomatoes were kept in the presence and absence of 1-MCP for 8 days and gene expression was examined by qRT-PCR. Expression was normalized to elongation factor-alpha control and is presented as relative to the untreated control. ETR1 and ACS are positive controls for ethylene-regulated gene expression. *RIN, NOR*, and *PSY1* are genes involved in the ripening of a fruit. Y-axis shows relative mRNA abundance and error bars were added based on SE from three replicates.

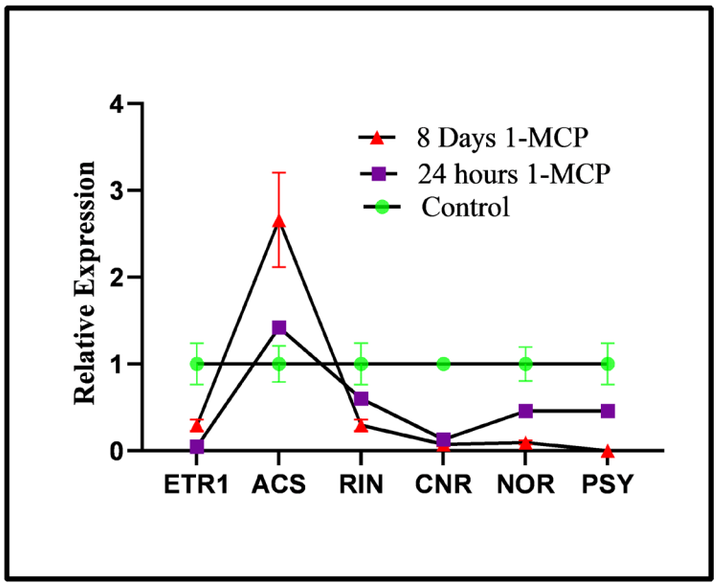
Asterisks show significant difference between control and 8-Days 1-MCP (* shows $p \leq 0.05$, ** shows $p \leq 0.01$)

FIGURE 5. Expression of ethylene signaling and ripening genes in 8 days 1-MCP treated tomatoes

of *ETR1* in 24 h and 8 days 1-MCP treated samples showed the similar pattern of expression (Figure 6). ETR1 gene downregulated in both treatments (Figure 6).

EXPRESSION OF *ACS* GENE IN TOMATO EXPOSED TO 1-MCP TREATMENT

ACS is involved in the biosynthetic pathway of ethylene (Pattyn, Vaughan-Hirsch & Van de Poel 2021). We analyzed the expression of *ACS* in 1-MCP treated samples. It showed an increase in its expression for both 24 h and 8 days of 1-MCP treatment (Figures 4 & 5). *ACS* is the second enzyme of the ethylene biosynthetic pathway and acts after SAM-Synthetase to convert S-Ado-Met into ACC (Pattyn, Vaughan-Hirsch & Van de Poel 2021). Comparative analysis of *ACS* in 24 h and 8 days treatment showed the similar pattern but high expression in 8 days treated samples (Figure 6).



Data shows the similar pattern of expression of genes in both 24 h and 8 days of 1-MCP treatment

FIGURE 6. Comparison of expression of ethylene signaling and ripening genes for 24 h and 8 days of 1- MCP treated tomatoes

TRANSCRIPT ANALYSIS OF RIPENING GENES OF TOMATOES TREATED WITH 1-MCP

Reduced gene expression was found for *PSY1, RIN, NOR,* and *CNR* in 24 h and 8 days of 1-MCP treated tomatoes as compared to control samples (Figures 4 & 5). Comparative analysis of *RIN*, *NOR,* and *PSY1* gene showed the similar pattern of expression in both 24 h and 8 days of treatment (Figure 6). 24 h treated samples showed insignificant higher level of expression comparatively (Figure 6).

DISCUSSION

In Pakistan, tomatoes demand exceeds the production rate due to their short shelf life, because of high daily consumption requirement it is therefore necessary to import tomatoes from other countries (Khokhar 2013). If we can enhance the tomatoes shelf life, then the import of tomatoes from other countries can be avoided. In this study, we have used 1-MCP to relegate the ripening procedure. 1-MCP inhibits fruit ripening by binding the ethylene receptors and turning down the ethylene response genes (Zhang et al. 2020). 1-MCP has more affinity than ethylene to bind to the ethylene receptors and inhibit the fruit ripening initiated by the ethylene (Zhang et al. 2020).

Treatment with 1-MCP delays the ripening process in tomatoes and can be stored for a longer period. Storage of good quality tomatoes treated with 1-MCP helps to decrease the tomato spoilage by over ripening (Taye et al. 2019). It also keeps the tomatoes fresh when transported to longer distances within the country (Kubota & Kroggel 2009). In this study, green unripe tomatoes were treated with 1-MCP for 24 h and kept in storage for 16 days. Colour change in 1-MCP treated tomatoes for 24 h delayed compared to the control tomatoes. Early softening in control tomatoes led to rotten tomatoes after the 16th day of storage, whereas 1-MCP treated tomatoes remained firm and fresh after the 16th day comparatively. 1-MCP treated tomatoes in comparison to control showed slow ripening process. Similarly, we saw a significant decrease in ripening when tomatoes were exposed to 1-MCP for longer duration. Green unripe tomatoes were treated with 1-MCP for 8 days in a container and kept in storage for 16 days after treatment. Longer exposure had a very significant effect on the colour of the tomatoes. All tomatoes retained the green colour under the influence of 1-MCP in the container and remained green until the 10th day of storage. Firmness of tomatoes was also maintained in 1-MCP treated tomatoes until the 16th day of storage. Control tomatoes changed the colour and firmness earlier

than 1-MCP treated samples. Longer exposure of 1-MCP in 8 days treated samples showed increased delay in colour change and firmness compared to the exposure of 1-MCP for 24 h. The weight of tomatoes indicates the delay in postharvest decay of tomatoes by maintaining the weight of tomatoes during the storage period.

Ripening continues in tomatoes as in other climacteric fruits due to the rise in endogenous ethylene levels (Taye et al. 2019). This climacteric peak is achieved with an increase in colour change, firmness, and expression of genes involved in the ripening process (Lelievre et al. 1997). Low shelf life of tomato has been associated with its great postharvest losses. Keeping them at low temperatures has not been effective enough to control the fruit loss (Kubota & Kroggel 2009). Therefore, there is a need to have some new tools that will target fruit ripening by either inhibiting or decreasing the ethylene production, which would ultimately reduce the fruit losses. More information is needed in the region regarding delayed ripening. The effectiveness of 1-MCP application to increase shelf life, quality, and freshness of fruit has been proven by many research labs, the main effects of which have been published (Blankenship & Dole 2003).

This study provides strong evidence that 1-MCP treatment delayed the fruit ripening process in tomato both at physiological and molecular levels. In daily consumption of fruits and vegetables, physiological parameters are of great importance. In the physiology of fruit, its colour and firmness are two important parameters in view of the consumer's acceptance (Distefano et al. 2022). Tomatoes have a very low shelf life because these parameters change rapidly. During the period of ripening, tomatoes undergo different developmental stages that include mature green, breaker, turning, pink, light red and red (Skolik et al. 2019). These physiological parameters are controlled by different levels of ethylene production during these developmental stages (Zhao, Nakano & Iwasaki 2021). Firmness is the most important factor considered in the postharvest and transport of fruits. Ethylene biosynthesis in fruits controls many physiological factors such as aging, nutrient contents, pigmentation, and softening. Application of 1-MCP to postharvest mango fruits delayed the aging and inhibited the softening of fruits by inhibiting the activity of the fruit softening enzymes (Li et al. 2020).

Results of transcript analysis are in accordance with the physiological results. Relative gene expression of ripening related genes was analyzed on the 1st day of storage in both 24 h and 8 days 1-MCP treated samples vs control group. Down regulation of *PSY1* gene in 1-MCP treated samples was seen compared to control. 1-MCP treatment causes a decrease in the expression of *PSY1*, our results were in coherent with Mir et al. (2004) as they demonstrated that 1-MCP treatment inhibits the ripening process by maintaining the firmness of the fruit along with the colour change and other physiological factors such as aroma and texture of fruit. One of the most important causes of deterioration of fruit quality is the weight loss of fruit and this is detrimental for the commercialization of fruit (Mir et al. 2004). Similarly plum fruit showed inhibition of decay when treated with 1-MCP (Valero et al. 2003). These results indicate that the treatment with 1-MCP can have a positive effect for a long shipment period of fruit and avoid economic losses. Effects of 1-MCP on postharvest decay of tomatoes were tested. Naturally and artificially infected fruits were subjected to 1-MCP at different concentrations and different time intervals. In these treated tomatoes, the occurrence of disease was significantly reduced because of the effects of 1-MCP on postharvest decay of fruits (Su & Gubler 2012).

Colourless non-ripening (*CNR*) is a SPB box transcription factors that monitor ripening in tomato (Gao et al. 2019). *CNR* expression was decreased in 1-MCP treated tomatoes for both 24 h and 8 days of treatment. However, control tomatoes showed an increase indicating ripening. *CNR* gene encodes a transcription factor for the regulation of genes involved in ripening. This gene is said to be a part of the epigenetic trigger of ripening in tomato (Eriksson et al. 2004). *CNR* expression decrease is associated with a colourless pericarp of fruit and hence taste and texture will also be affected if it is silenced completely (Fujisawa & Ito 2013). *RIN* is a MADS box transcription factor found in tomatoes that control the ripening process in tomatoes with ethylene. It is found that ethylene is required for the expression of *RIN* in tomatoes and the other *RIN* targeted genes in the tomato (Fujisawa & Ito 2013). *NOR* is also the transcription factors of MADS-box regulatory network. In this study, 1-MCP treatment decreased the expression of *RIN* and *NOR*. Gene expression of *RIN, CNR, NOR,* and *PSY* was decreased in 1-MCP treated tomatoes, which indicates that these genes are regulated by the ethylene. As 1-MCP inhibits the perception and signaling of ethylene (Street et al. 2015). Reduction in the expression of ripening related genes *RIN, CNR, NOR,* and *PSY* indicates that they are either directly or indirectly controlled by ethylene (Yokotani et al. 2009).

In our study, there was a marked decrease in *ETR* mRNA levels in 1-MCP treated samples compared to control. Ethylene biosynthetic and signal transduction pathway genes were greatly reduced in response to 1-MCP treatment. Gene expression of ethylene receptors, *ETR2*, *ERS2*, *ETR4,* and *ETR5* was reduced in 1-MCP treatment (Gamrasni et al. 2020). Ethylene receptor expression was checked for *ETRa* receptors in apples. Gene expression of ethylene receptors *Md-ETR1* and *Md-ERS1* in apples was down regulated in response to 1-MCP treatment (Dal Cin et al. 2006).

Expression level of *ACS* gene, which is an important enzyme of ethylene biosynthetic pathway, we observed increase in mRNA level of both 24 h and 8 days of 1-MCP treated samples. *ACS* is a biosynthetic pathway gene for ethylene, its biosynthesis is triggered by ethylene signaling pathway (Pattyn, Vaughan-Hirsch & Van de Poel 2021), but the biosynthesis of *ACS* is also controlled by other hormones and acts upstream of the ethylene signaling pathway. Ethylene biosynthesis is also controlled by other hormones like Auxin, Cytokinin, Abscisic acid, Jasmonate, and Brassinoids (Pattyn, Vaughan-Hirsch & Van de Poel 2021). Whereas 1-MCP inhibits the ethylene receptors and inhibits the downstream signaling pathway (Street et al. 2015).

Yokotani et al. (2009) reported the increased expression of *Le ACS2* and *Le ACS4* genes in ethylene insensitive tomatoes. 1-MCP treatment could not reduce the expression of *ASC* genes in tomatoes. Rise in *ASC* genes is due to the ethylene independent expression of these genes. There is a cross talk between auxins, gibberellins, and other plant hormones that ultimately lead to an increase in ethylene production. Enzymes that are expressed and translated before the application of 1-MCP exert their effects on ripening processes (Mir et al. 2004). Expression analysis of berry, guava, and Arabidopsis has also provided similar results. They had *ACS* induction due to high levels of auxin (Abel et al. 1995; Yoshii & Imaseki 1982). Taken together this data indicates that there is a remarkable delay in ripening of tomato fruit after its treatment with 1-MCP. Our physiological data is in accordance with the molecular data. Molecular results showing the down-regulation of ripening genes and physiological data showing phenotypes of delayed ripening. However, if we compare our 24 h 1-MCP treated results with 8 days 1-MCP treated results, it shows that the molecular results for both sets are almost the same as both were tested on the 1st day of storage after they were taken out of the 1-MCP container. However, the physiological results show that the longer exposure of 1- MCP (8 days) to tomatoes shows increased delay in fruit ripening compared to shorter exposure, that is, 24 h. This is because the ethylene receptors are degraded after some time (Kevany et al. 2007). New receptors are synthesized which are then occupied by ethylene in post 1-MCP treatment. Whereas longer exposure of 1-MCP does not allow ethylene to bind to the newly synthesized receptors (In et al. 2013). Having enough information of 1-MCP effect on ripening delay will eventually help in commercializing this method for tomato fruit's delayed ripening.

Conclusions

Efficaciousness of 1-MCP as an alternative technology is shown based on physiological and molecular analysis. It shows the inhibitory effect of MCP in 24 h (short exposure) and 8 days of 1-MCP treatment (long exposure) in comparison to control (non-treated) for delayed ripening of Tomato (*Lycopersicum esculentum*) fruits in Pakistan. Down regulation of *RIN, NOR, CNR,* and *PSY 1* in the presence of 1-MCP, shows that these transcription factors are ethylene dependent. 1-MCP might not be effective for all horticultural commodities, but it surely has a positive impact on the delayed ripening of fruits. Finally, treating fruits with 1-MCP can prevent their damage and loss during shipment and hence will prove economical and minimize future postharvest losses.

Acknowledgements

All authors declare no affiliation with any organization, no involvement, or entity with any financial or non-financial interest. All work mentioned in the manuscript is done independently.

References

Abel, S., Nguyen, M.D., Chow, W. & Theologis, A. 1995. ASC4, a primary indoleacetic acid-responsive gene encoding 1-Aminocyclopropane-1-carboxylate synthase in *Arabidopsis thaliana* structural characterization, expression in *Escherichia coli*, and expression characteristics in response to auxin. *Journal of Biological Chemistry* 270(32): 19093-19099.

Abeles, F.B., Morgan, P.W. & Saltveit Jr., M.E. 2012. *Ethylene in Plant Biology*. 2nd ed. San Diego: Academic Press.

Batu, A. 2004. Determination of acceptable firmness and colour values of tomatoes. *Journal of Food Engineering* 61(3): 471-475.

Biswas, P., East, A., Hewett, E. & Heyes, J. 2014. Ripening delay caused by 1-MCP may increase tomato chilling sensitivity. *New Zealand Journal of Crop and Horticultural Science* 42(2): 145-150.

Blankenship, S.M. & Dole, J.M. 2003. 1-Methylcyclopropene: A review. *Postharvest Biology and Technology* 28(1): 1-25.

Blankenship, S.M. & Sisler, E.C. 1993. Response of apples to diazocyclopentadiene inhibition of ethylene binding. *Postharvest Biology and Technology* 3(2): 95-101.

Chang, K.N., Zhong, S., Weirauch, M.T., Hon, G., Pelizzola, M., Li, H., Huang, S.S.C., Schmitz, R.J., Urich, M.A. & Kuo, D. 2013. Temporal transcriptional response to ethylene gas drives growth hormone cross-regulation in Arabidopsis. *Elife* 2: e00675.

Dal Cin, V., Rizzini, F.M., Botton, A. &Tonutti, P. 2006. The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit. *Postharvest Biology and Technology* 42(2): 125-133.

Distefano, M., Mauro, R.P., Page, D., Giuffrida, F., Bertin, N. & Leonardi, C. 2022. Aroma volatiles in tomato fruits: The role of genetic, preharvest and postharvest factors. *Agronomy* 12(2): 376.

Eriksson, E.M., Bovy, A., Manning, K., Harrison, L., Andrews, J., De Silva, J., Tucker, G.A. & Seymour, G.B. 2004. Effect of the colorless non-ripening mutation on cell wall biochemistry and gene expression during tomato fruit development and ripening. *Plant Physiology* 136(4): 4184-4197.

Fraser, P.D., Enfissi, E.M.A., Halket, J.M., Truesdale, M.R., Yu, D., Gerrish, C. & Bramley, P.M. 2007. Manipulation of phytoene levels in tomato fruit: Effects on isoprenoids, plastids, and intermediary metabolism. *The Plant Cell* 19(10): 3194-3211.

Frusciante, L., Carli, P., Ercolano, M., Pernice, R., Di Matteo, A., Fogliano, V. & Pellegrini, N. 2007. Antioxidant nutritional quality of tomato. *Molecular Nutrition & Food Research* 51: 609-617.

Fujisawa, M. & Ito, Y. 2013. The regulatory mechanism of fruit ripening revealed by analyses of direct targets of the tomato MADS-box transcription factor ripening inhibitor. *Plant Signaling & Behavior* 8(6): 371-386.

Gamrasni, D., Feldmesser, E., Ben-Arie, R., Raz, A., Tabatznik Asiag, A., Glikman, M., Aharoni, A. & Goldway, M. 2020. Gene expression in 1-Methylcyclopropene (1-MCP) treated tomatoes during pre-climacteric ripening suggests shared regulation of methionine biosynthesis, ethylene production and respiration. *Agronomy* 10(11): 1669.

Gao, Y., Zhu, N., Zhu, X., Wu, M., Jiang, C.Z., Grierson, D., Luo, Y., Shen, W., Zhong, S., Fu, D-Q. & Qu, G. 2019. Diversity and redundancy of the ripening regulatory networks revealed by the fruit encode and the new CRISPR/Cas9 *CNR* and *NOR* mutants. *Horticulture Research* 6(1): 39.

Giorio, G., Stigliani, A.L. & D'Ambrosio, C. 2008. Phytoene synthase genes in tomato (*Solanum lycopersicum* L.) - new data on the structures, the deduced amino acid sequences and the expression patterns. *The FEBS Journal* 275(3): 527-535.

Giovannoni, J.J. 2004. Genetic regulation of fruit development and ripening. *The Plant Cell* 16(suppl 1): S170-S180.

Huber, D.J., Jeong, J. & Ritenour, M. 2003. Use of 1-Methylcyclopropene (1-MCP) on tomato and avocado fruits: Potential for enhanced shelf life and quality retention. Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences. University of Florida.

In, B.C., Strable, J., Binder, B.M., Falbel, T.G. & Patterson, S.E. 2013. Morphological and molecular characterization of ethylene binding inhibition in carnations. *Postharvest Biology and Technology* 86: 272-279.

Kevany, B.M., Tieman, D.M., Taylor, M.G., Cin, V.D. & Klee, H.J. 2007. Ethylene receptor degradation controls the timing of ripening in tomato fruit. *The Plant Journal* 51(3): 458-467.

Khokhar, K.M. 2013. Present status and prospects of tomatoes in Pakistan. *Agriculture Corner-Farmers to Global Market.* pp. 1-21.

Kubota, C. & Kroggel, M. 2009. Application of 1-MCP for long distance transportation of high quality tomato seedlings. *V International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops* 898: 279-285.

Lelievre, J-M., Tichit, L., Dao, P., Fillion, L., Nam, Y-W., Pech, J.C. & Latché, A. 1997. Effects of chilling on the expression of ethylene biosynthetic genes in Passe-Crassane pear (*Pyrus communis* L.) fruits. *Plant Molecular Biology* 33(5): 847-855.

Li, L., Li, C., Sun, J., Sheng, J., Zhou, Z., Xin, M., Yi, P., He, X., Zheng, F. &Tang, Y. 2020. The effects of 1-methylcyclopropene in the regulation of antioxidative system and softening of mango fruit during storage. *Journal of Food Quality* 2020: Article ID. 6090354.

Li, S., Zhu, B., Pirrello, J., Xu, C., Zhang, B., Bouzayen, M., Chen, K. &Grierson, D. 2020. Roles of RIN and ethylene in tomato fruit ripening and ripening-associated traits. *New Phytologist* 226(2): 460-475.

Liu, M., Pirrello, J., Chervin, C., Roustan, J.P. & Bouzayen, M. 2015. Ethylene control of fruit ripening: Revisiting the complex network of transcriptional regulation. *Plant Physiology* 169(4): 2380-2390.

Manning, K., Tör, M., Poole, M., Hong, Y., Thompson, A.J., King, G.J., Giovannoni, J.J. & Seymour, G.B. 2006. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nature Genetics* 38(8): 948-952.

Mir, N., Canoles, M., Beaudry, R., Baldwin, E. & Mehla, C.P. 2004. Inhibiting tomato ripening with 1-methylcyclopropene. *Journal of the American Society for Horticultural Science* 129(1): 112-120.

Moretti, C.L., Araujo, A.L., Marouelli, W.A. & Silva, W.L.C. 2002. 1-Methylcyclopropene delays tomato fruit ripening. *Horticultura Brasileira* 20(4): 659-663.

Mursalat, M., Rony, A.H., Rahman, A., Islam, M. & Khan, M.S. 2013. A critical analysis of artificial fruit ripening: Scientific, legislative and socio-economic aspects. *ChE Thoughts* 3(1): 1-7.

Pattyn, J., Vaughan-Hirsch, J. & Van de Poel, B. 2021. The regulation of ethylene biosynthesis: A complex multilevel control circuitry. *The New Phytologist* 229(2): 770-782.

Rao, A.V. & Agarwal, S. 1998. Bioavailability and *in vivo* antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. *Nutr. Cancer* 31(3): 199-203.

Rizvi, J. 2017. Tomato being imported from Iran, Afghanistan, not India. *The News.* September 26, 2017.

Sisler, E.C. 2006. The discovery and development of compounds counteracting ethylene at the receptor level. *Biotechnology Advances* 24(4): 357-367.

Sisler, E.C. & Serek, M. 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiologia Plantarum* 100(3): 577-582.

Skolik, P., Morais, C.L.M., Martin, F.L. & McAinsh, M.R. 2019. Determination of developmental and ripening stages of whole tomato fruit using portable infrared spectroscopy and Chemometrics. *BMC Plant Biology* 19(1): 236.

Street, I.H., Aman, S., Zubo, Y., Ramzan, A., Wang, X., Shakeel, S.N., Kieber, J.J. & Schaller, G.E. 2015. Ethylene inhibits cell proliferation of the Arabidopsis root meristem. *Plant Physiology* 169(1): 338-350.

Su, H. & Gubler, W.D. 2012. Effect of 1-methylcyclopropene (1-MCP) on reducing postharvest decay in tomatoes (*Solanum lycopersicum* L.). *Postharvest Biology and Technology* 64(1): 133-137.

Taye, A.M., Tilahun, S., Seo, M.H., Park, D.S. & Jeong, C.S. 2019. Effects of 1-MCP on quality and storability of cherry tomato (*Solanum lycopersicum* L.). *Horticulturae* 5(2): 29.

Valero, D., Martınez-Romero, D., Valverde, J., Guillen, F. & Serrano, M. 2003. Quality improvement and extension of shelf life by 1-methylcyclopropene in plum as affected by ripening stage at harvest. *Innovative Food Science & Emerging Technologies* 4(3): 339-348.

Wang, R., Lammers, M., Tikunov, Y., Bovy, A.G., Angenent, G.C. & de Maagd, R.A. 2020. The rin, nor and Cnr spontaneous mutations inhibit tomato fruit ripening in additive and epistatic manners. *Plant Science* 294: 110436.

Yokotani, N., Nakano, R., Imanishi, S., Nagata, M., Inaba, A. & Kubo, Y. 2009. Ripening-associated ethylene biosynthesis in tomato fruit is autocatalytically and developmentally regulated. *Journal of Experimental Botany* 60(12): 3433-3442.

Yoshii, H. & Imaseki, H. 1982. Regulation of auxin-induced ethylene biosynthesis. Repression of inductive formation of 1-aminocyclopropane-1-carboxylate synthase by ethylene. *Plant and Cell Physiology* 23(4): 639-649.

Zhang, J., Ma, Y., Dong, C., Terry, L.A., Watkins, C.B., Yu, Z. & Cheng, Z.M.M. 2020. Meta-analysis of the effects of 1-methylcyclopropene (1-MCP) treatment on climacteric fruit ripening. *Horticulture Research* 7(1): 208.

Zhao, T., Nakano, A. & Iwasaki, Y. 2021. Differences between ethylene emission characteristics of tomato cultivars in tomato production at plant factory. *Journal of Agriculture and Food Research* 5: 100181.

*Corresponding author; email: snq28@yahoo.com