Influence of 1-MCP on texture, related enzymes, quality and their relative gene expression in ‘Amrapali’ mango (*Mangifera indica* L.) fruits

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Abstract The mango fruits remain biologically active even after harvest as they continue respiration, transpiration and other bio-chemical processes. Being highly perishable, the fruit quality deteriorates fast under ambient conditions (30 ± 5 °C and $50 \pm 5\%$ RH), rendering them unmarketable within 5–6 days. In order to extend the shelf-life of ‘Amrapali’ mango fruits, we have treated them with three different concentrations (500, 750 and 1000 ppb) of 1-Methylcyclopropene (1-MCP) @ 20 °C and stored at ambient conditions. Among all the treatments, 1000 ppb was found to be an effective in extending shelf-life till twelfth day with minimum physiological loss in weight (19.24%), maximum firmness (10.43 N), highest retention of quality parameters such as soluble solid concentrates (27.88 °B), ascorbic acid (28.49 mg 100 g⁻¹ FW) and total antioxidant activity (675.41 µmol Trolox g⁻¹ FW) compared to untreated mango fruits (21.79%, 5.45 N, 23.17 °B, 19.55 mg 100 g⁻¹ FW and 265.41 µmol Trolox g⁻¹ FW, respectively). Gene expression studies have revealed that the texture related gene *expansin* was significantly

repressed till fifth day of storage with increasing concentrations of 1-MCP.

Keywords 1-MCP · Mango · Texture · Postharvest · Gene expression · *Expansin*

Introduction

Mango (*Mangifera indica* L.) is an economically important fruit crop of India, known for its delicious taste, exceptional flavour, and high nutritive value. Based on its popularity in the masses, wide adaptability, varietal diversity and attractive appearance, it is appropriately titled as ‘King of fruits’ in India (Litz 2009). Despite being a nutritive fruit, its availability in the market is greatly hindered by short postharvest life. Under ambient conditions, these fruits keep well only for 5–6 days as they remain biologically active even after harvest and carry out respiration, transpiration and other bio-chemical processes, which deteriorate fruit quality and finally making it unmarketable. Hence, it is essential to adopt an appropriate integrated system of postharvest technology, right from harvesting, till it reaches the end consumers (Singh et al. 2013). Several postharvest treatments viz. pre-cooling, hot water treatment, refrigeration, edible skin coatings, wax coatings, ethylene absorbents, ethylene synthesis and action inhibitors, chemicals such as growth regulators and fungicides are in vogue for enhancing the shelf life of fruits (Gill et al. 2005). Yet research is going on to find still better approaches for extending the shelf life and maintaining the postharvest quality of fruits during storage and subsequent marketing.

The climacteric fruits such as mango are characterized by a sudden upsurge in ethylene production during ripening

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and thus become susceptible to severe postharvest losses due to rapid ripening, triggered by ethylene (Wills et al. 2007). Due to high perishable nature, less than one per cent fruits get exported and 30–40% of the produce goes waste as a result of faulty or improper handling during transportation and storage. Reduction of such losses can improve farmers' income and per capita consumption. However, the major mango producing countries still lack commercial scale postharvest handling facilities for cooling, cold storage, quarantine treatments and other cutting edge technologies developed especially for mango (Singh and Singh 2012).

The postharvest quality of the fruits can be easily manipulated to certain extent with the application of various compounds. In the recent times, postharvest application of 1-methylcyclopropene (1-MCP), has emerged as a ray of hope for extending the shelf life and quality of some fruits at ambient conditions. This compound exhibits positive responses in the treated fruits, and has emerged as one of the wonder chemicals in fruit industry. It acts on ethylene biosynthesis and its perception in the fruits during ripening (Sisler and Blankenship 1996; Watkins 2006). 1-methylcyclopropene (1-MCP) was invented by Sisler and Blankenship during the year 1996 and it was later designated as a wonder chemical for postharvest use. It is regarded as ethylene action inhibitor and greatly helps in extending the shelf life and quality of fresh horticultural produce. The affinity of 1-MCP for the receptor is approximately 10 times greater than that of ethylene (Sisler and Serek 1997). It is active at much lower concentrations compared to ethylene. It also influences ethylene biosynthesis in some species through feedback inhibition (Blankenship and Dole 2003).

Expansins are a kind of cell wall proteins that play an important role in cell wall disassembly of non-growing tissues viz. ripening fruits. They are said to involve in the disruption of hydrogen bonds between cellulose microfibrils and crosslinking wall glycosides (McQueen-Mason and Cosgrove 1995). The expression of *Expansin* genes during various stages of fruit ripening in peach (Hayama et al. 2003; Obenland et al. 2003), pear (Hiwasa et al. 2003), banana (Harrison et al. 2001) and strawberry (Trivedi and Nath 2004) suggests their important role in regulating textural changes during fruit senescence. Since, this gene is rapidly triggered by ethylene, it may be a good candidate for manipulation of softening in mango via recombinant DNA technology. Keeping in view the above mentioned gaps, facts and the available opportunities in mind, the present research work has been undertaken to extend the marketability and enhance the shelf life of 'Amrapali' mango fruits at ambient conditions ($30 \pm 5^\circ\text{C}$ and $50 \pm 5\%$ RH). Also, to understand the textural changes at molecular level, gene expression profiling was done

at specified intervals after the treatment to get a deeper insights at gene level.

Materials and methods

Plant material and treatments

The current research work was carried out in the Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi, during the year 2012–2015. Gene expression studies were carried out at ICAR-National Research Center for Plant Biotechnology, New Delhi. The mature green 'Amrapali' mango fruits were harvested manually from the experimental orchards of IARI, New Delhi, along with a little stalk and were transported immediately to the laboratory. Later the fruits were then desapped, cleaned with tap water and air-dried for 10–15 min. Then, the fruits were placed in air tight plastic containers of 11 L volume, in which, a pre-calculated amount of 1-MCP (AgroFreshTM) powder was placed and dissolved using distilled water. Later, the containers were closed airtight using parafilm tape and incubated for 24 h at 20°C . After 24 h of exposure, the fruits were removed from the containers and packed in the ventilated CFB boxes and stored at ambient conditions ($30 \pm 5^\circ\text{C}$ and $50 \pm 5\%$ RH). In total, 100 fruits per treatment with four replications of 25 each were taken for the study. The mango fruits were sampled for physical, physiological and biochemical parameters at 3 day's interval, respectively. The treatments attempted in this experiment were 1-Methylcyclopropene (500 ppb), 1-Methylcyclopropene (750 ppb), and 1-Methylcyclopropene (1000 ppb) along with control.

Observations recorded

Fruit firmness

The firmness of mango fruit, at equatorial region, was measured as the force required for puncturing the fruits using a texture analyzer (Model: TA + Di, Stable Microsystems, UK). A probe of 2 mm diameter was used, set at a cross head speed of 0.5 mm s^{-1} with using a 500 kg load cell. The firmness was defined in terms of maximum force (kgf) during puncturing and was expressed in Newtons (N). The first peak in the force deformation curve was taken as firmness of the fruit.

Physiological loss in weight (PLW)

Individual mango fruits were marked in each treatment to record the physiological loss in weight. The weight of the

individual fruit was recorded using high precision electronic balance before storage. Thereafter, the fruit weights were recorded regularly during storage and the cumulative PLW was calculated with the following formula:

$$\text{PLW (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Respiration rate

The respiration rate of the treated and untreated mango fruits were measured in auto gas analyzer (Make: Checkmate 9900 O₂/CO₂, Denmark) and calculated as under:

$$\begin{aligned} \text{Respiration rate (mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}) \\ = \frac{\text{CO}_2 \text{ (\%)} \times \text{Head space}}{100 \times \text{Weight (kg)} \times \text{Time (h)}} \end{aligned}$$

Ethylene evolution rate

For determination of ethylene evolution rate in treated and untreated mango fruits, two fruits from each replication were weighted and kept inside specially designed airtight container (150 mL capacity) provided with a sub seal septum for gas sampling. One mL gas sample was drawn through the sub seal septum with the help of a gas tight micro syringe after a specified time of trapping of fruit. The sample was injected into the GC and concentration of evolved ethylene (ppm) within the time interval was recorded from the integrator, and the rate of ethylene evolution was expressed as $\mu\text{L kg}^{-1} \text{ h}^{-1}$.

Total antioxidant activity

Total antioxidant capacity of the mango fruit pulp was determined using CUPRAC method, standardized by Apak et al. (2004) with slight modifications and expressed as $\mu\text{mol Trolox g}^{-1} \text{ FW}$.

Determination of pectin methylesterase (PME) activity

Pectin methylesterase (PME) activity in mango fruit pulp was measured following the method of Hagerman and Austin (1986) with minor modifications (Sharma et al. 2012a, b). The method is based on the colour change of a pH indicator during the PME catalysed reaction. In a cuvette, 2.0 mL of pectin (0.5%) is mixed with 0.15 mL of bromothymol blue (0.01%) and 0.83 mL of water. The absorbance of the mixture is read against water as blank at 620 nm. A constant value of A₆₂₀ at this stage indicates

nonexistence of non-enzymatic hydrolysis. The reaction is started by adding 50 μL of enzyme solution and the rate of decrease in A₆₂₀ was recorded. The acid produced by PME action lowers the pH of the medium and thereby cause protonation of the indicator dye to produce a change in absorbance at 620 nm. The change in absorbance is continuously monitored spectrophotometrically and the initial rate of reaction is determined. A standard graph is plotted (OD vs. time) using different known concentrations of glacial acetic acid and the rate of reaction is determined from the linear portion of the graph. The PME activity was expressed as $\mu\text{mol min}^{-1} \text{ g}^{-1} \text{ FW}$.

Estimation of soluble solids concentrates

The mango fruits were pulped for juice extraction and the juice was used for determination of total soluble solids by using a hand refractometer. The values were corrected at 20 °C and expressed as °B.

Determination of ascorbic acid content

Ascorbic acid content of mango pulp samples was determined by 2, 6-dichlorophenol indophenol visual titration method described by Ranganna (1986). Five milliliter of filtered sample extracted in 3% metaphosphoric acid along with 5 mL of 3% metaphosphoric acid was taken in a conical flask and titrated against the standard dye solution. The end point was light pink colour, which persisted for 10 s. The dye solution was standardized using standard ascorbic acid for determination of dye factor. Finally the ascorbic acid content was calculated using the formula

$$\begin{aligned} \text{Ascorbic acid (mg 100g}^{-1}) \\ = \frac{\text{Titre value} \times \text{Dye factor} \times \text{Volume made up}}{\text{Volume taken} \times \text{Weight of the sample}} \times 100 \end{aligned}$$

Gene expression study

Sampling was done at specified intervals after the treatment and the samples were frozen in liquid nitrogen before storage at -80°C for isolation of RNA. Total RNA was isolated using the protocol developed by Reddy et al. (2015). The quality of the isolated RNA was evaluated using TBE gel electrophoresis and the quantity was estimated spectrophotometrically using a nano spectrophotometer (Thermo-Fisher Scientific, Wilmington, DE, USA). Later cDNA was synthesized from it and the targeted gene expression was noticed in a PCR using the self designed primers as shown below:

Primer sequences and their respective annealing temperatures

Sl. no.	Gene specific primers	Primer sequence	Annealing temperature (Tm)
1	<i>MExpn</i>	Forward: 5'TGCCATGCC CATGTTTCT 3'	54 °C
		Reverse: 5'CGACGAATGT TTGACCGAATTG 3'	

Finally, the PCR products were run on 1.2% agarose gel along with 100 bp DNA ladder (Molecular marker) and observed under UV light in a gel documentation unit. After the confirmation of gene expression from genomic as well as cDNA, the gene expression was verified in triplicates and the quantification of the gene expression was calculated using the software (© Gene tools, Syngene Bio-imaging Pvt. Ltd., Cambridge, UK).

Statistics

The experiment was designed in a factorial completely randomized design (CRD) with three replications. The data was analyzed as per the design and the results were compared from the CD value obtained through ANOVA (Panse and Sukhatme 1984).

Results and discussion

Physiological loss in weight

The physiological loss in weight occurs mainly through the respiration and transpiration losses along with some other metabolic processes. Less loss of moisture from the produce indicates the maintenance of turgidity which in turn justifies the freshness of the produce. Here, the physiological loss in weight (PLW) of the mango fruits increased with the advancement of storage period, rather slowly in the beginning but at a faster pace as the storage period advanced (Table 1). Furthermore, 1-MCP treatments have significantly lowered physiological weight loss of mango fruits during storage. The PLW was minimum (7.88%) in the mango fruits treated with 1-MCP (1000 ppb) followed significantly (8.28%) by 1-MCP (750 ppb) treatment and maximum PLW (10.6%) was observed in the untreated mango fruits. Also, the interaction effect between treatment and storage period ($T \times S$) was highly significant as the highest PLW (21.79%) was observed in untreated mango fruits on twelfth day while the lowest PLW (4.24%) was recorded in mango fruits treated with 1-MCP (1000 ppb) on third day of storage (Table 1). The reduced weight loss

in treated fruits can be attributed to low levels of respiration rate and delayed ripening of such fruits which maintained rigidity of the fruit tissue and thereby reduced the transpiration losses. Similar favorable effects of 1-MCP in reducing the PLW was also reported in 'Royal Zee' plums (Dong et al. 2002), 'Santa Rosa' plums (Sharma et al. 2012b), 'Allison' kiwifruits (Jhalegar et al. 2012) and mango fruits (Burondkar et al. 2013).

Fruit firmness

The firmness of fruit tissues is mainly due to the physical properties of the individual cell walls and the middle lamella that contains the pectic-substances which act as cementing material. During ripening, the fruit tissues become soft due to the degradation of cell wall and inter-cellular adhesive substances. Hence, firmer fruits stay long in the market than those exhibiting less firmness. In the current study, firmness of 'Amrapali' mango fruits has decreased gradually with simultaneous softening of fruits with increase in storage period. Mango fruits exposed to 1-MCP treatment (1000 ppb) were firm, exhibiting significantly higher texture (18.72 N) than other concentrations of 1-MCP or untreated fruits (14.92 N) (Table 1). The remarkable influence of 1-MCP treatment on fruit softening might be due to reduction in ethylene in such fruits as ethylene is extensively involved in postharvest fruit softening and acceleration of overall ripening of fruits. Hence, 1-MCP induced ethylene inhibition would have caused a delay in fruit softening during storage. The rapid fruit softening in untreated fruits can be associated with solubilization of pectin and disruption of the xyloglucan–cellulose microfibril networks in the mango fruit moderated through increased activity of exo-polygalacturonase (PG), pectin methylesterase, $\beta(1 \rightarrow 4)$ -glucanase and β -galactosidase (Ali et al. 2004). The retardation of fruit softening in response to 1-MCP treatment has also been reported in several other fruits such as guava (Bassetto et al. 2005), plum (Sharma et al. 2012a, b), avocado (Pesis et al. 2002), kiwifruit (Jhalegar et al. 2012) and papaya (Manenoi et al. 2007).

Respiration rate

In the current study, the respiration rate of the 'Amrapali' mango fruits have shown a gradual increase during ripening and senescence up to certain period and then it decreased slowly. The highest respiratory peak (363.48 mL $\text{CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) was observed in the untreated mango fruits on the sixth day of storage while the climacteric peak was suppressed (336.68 mL $\text{CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) and deferred till ninth day of storage in the 1-MCP (750 and 1000 ppb) treated fruits (Fig. 1a). The delay and suppression of

Table 1 Effect of postharvest application of ethylene action inhibitor, 1-MCP on fruit firmness (N) and physiological loss in weight (%) of ‘Amrapali’ mango fruits during storage at ambient conditions (30 ± 5 °C and $50 \pm 5\%$ RH)

Treatment	Fruit firmness Storage period (days)						Physiological loss in weight (%) Storage period (days)					
	0 day	3 days	6 days	9 days	12 days	Mean	0 day	3 days	6 days	9 days	12 days	Mean
1-MCP (500 ppb)	28.92	23.60	13.77	8.69	7.57	16.51	0.00	4.85	7.04	11.52	19.91	8.66
1-MCP (750 ppb)	28.92	23.75	14.65	10.22	8.79	17.27	0.00	4.39	6.46	10.72	19.83	8.28
1-MCP (1000 ppb)	28.92	25.85	16.13	12.26	10.43	18.72	0.00	4.24	6.22	9.68	19.24	7.88
Control	28.92	20.31	11.78	8.14	5.45	14.92	0.00	6.39	10.46	14.35	21.79	10.60
Mean	28.92	23.38	14.08	9.83	8.06		0.00	4.97	7.55	11.57	20.19	
	C.D.	SE (d)	SE (m)				C.D.	SE (d)	SE (m)			
Treatment (T)	0.54	0.27	0.19				0.20	0.10	0.07			
Storage period (S)	0.60	0.30	0.21				0.23	0.11	0.08			
T $\times$ S	1.21	0.60	0.43				0.45	0.23	0.16

Fig. 1 Influence of postharvest application of ethylene action inhibitor (1-MCP) on respiration rate ($\text{mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) (a) and ethylene evolution rate ($\mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$) (b) of ‘Amrapali’ mango fruits stored at ambient conditions (30 ± 5 °C and $50 \pm 5\%$ RH)

respiratory rates in response to 1-MCP treatment, was also reported in other climacteric fruits such as guava (Singh and Pal 2008), avocado (HersHKovitz et al. 2005), papaya (Manenoi et al. 2007) and plums (Sharma et al. 2012b)

where 1-MCP suppressed and delayed respiratory peaks during fruit ripening.

Ethylene evolution rate

The ethylene produced internally, regulates the major changes during ripening of climacteric fruits, such as peel and pulp colour, firmness, soluble solid content, acidity and respiratory rate. We have observed that the ethylene production rate has increased initially but declined thereafter with storage. There is significant effect of 1-MCP on the ethylene evolution rate of ‘Amrapali’ mango fruits as the ethylene peaks were deferred by 3 days compared to the untreated fruits. However, there was no significant difference in the amounts of ethylene produced during ripening. Relatively maximum ethylene production ($1.62 \mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$) was observed in mango fruits treated with 1-MCP @ 500 ppb on ninth day of storage followed by the untreated fruits ($1.58 \mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$) on the sixth day of storage (Fig. 1b). While the lowest climacteric peak of ethylene was observed in the mango fruits treated with 1-MCP @ 1000 ppb on the sixth day of storage. The greater delay in the ethylene production by 1-MCP treatment might be due to interference of 1-MCP with the autocatalytic production of ethylene, as all ethylene binding sites might have been irreversibly blocked by 1-MCP. Similar reports of delayed ethylene production with application of 1-MCP have been documented in many other fruit crops (Blankenship and Dole 2003).

Total antioxidant activity

Natural antioxidants, particularly of fruits and vegetables, have gained increasing interest among consumers and the scientific community because their regular consumption

can lower the risk of cardiovascular diseases and cancer. All the treatments have significantly influenced the total antioxidant activity of the mango fruits during storage which progressively increased up to certain extent and declined gradually thereafter. Irrespective of the storage period, the total antioxidant activity was maximum ($692.47 \mu\text{mol Trolox g}^{-1}$ Fresh Weight) in the fruits treated with 1-MCP @ 1000 ppb on ninth day followed by the same treatment ($675.41 \mu\text{mol Trolox g}^{-1}$ FW) on twelfth day of storage and the minimum antioxidant activity ($224.68 \mu\text{mol Trolox g}^{-1}$ FW) was observed on the day of harvest (Fig. 2a). This higher antioxidant activity of the 1-MCP treated fruits might be due to slower ripening and greater retention of phenolic compounds and ascorbic acid. These results are in line with the reports of Singh and Dwivedi (2008) who reported reduced levels of H_2O_2 and lipid peroxidation, concomitant with increased catalase and superoxide dismutase activities. The reduction in total antioxidant activity towards the end of storage period can be directly correlated to the reduction of ascorbic acid content towards the end of storage life.

Fig. 2 Changes in total antioxidant ($\mu\text{mol Trolox g}^{-1}$) activity (a) and pectin methyl esterase ($\mu\text{mol min}^{-1}$) activity (b) in 'Amrapali' mango fruit fumigated with ethylene action inhibitor 1-MCP and stored at ambient conditions ($30 \pm 5^\circ\text{C}$ and $50 \pm 5\%$ RH)

Pectin methylesterase (PME) activity

The PME activity has increased gradually for some time and then it showed declining trend during storage. However, the rate of increase was significantly high in the untreated mango fruits compared to the treated ones (Fig. 2b). The PME activity of untreated mango fruits reached maximum ($0.248 \mu\text{mol min}^{-1}$) on the sixth day of storage while the fruits fumigated with 1-MCP (500 and 750 ppb) have shown maximum (0.218 and $0.222 \mu\text{mol min}^{-1}$) on the ninth day of storage. However, the PME activity continually increased but at a slower pace in the mango fruits treated with 1-MCP @ 1000 ppb (Fig. 2b). The reduced PME activity in the 1-MCP treated mango fruits can be attributed to retarding effects of 1-MCP on the fruit softening. Our results find the support from the reports of Lohani et al. (2004), who found lowered PG and PME enzyme activities in 1-MCP treated banana compared to the untreated fruits. Similar effects of 1-MCP on PME activity of plum (Khan and Singh 2007; Sharma et al. 2012b) and papaya (Ahmad et al. 2013) have also been reported.

Fig. 3 Changes in the soluble solids concentrates (°B) (a) and ascorbic acid content ($\text{mg } 100 \text{ g}^{-1}$ FW) (b) in 'Amrapali' mango fruits treated with ethylene action inhibitor (1-MCP) after harvest and stored at ambient conditions ($30 \pm 5^\circ\text{C}$ and $50 \pm 5\%$ RH)

Fig. 4 Comparative transcript expression profiling of *MiExpA1* gene in ‘Amrapali’ mango fruits after 4, 24, 48 h, 5 and 7 days of 1-MCP treatment and storage at ambient conditions. For relative quantification, the control (T₄) was taken as 1 and the relative fold changes in expression were calculated for other treatments. [T₁: 500 ppb, T₂: 750 ppb, T₃: 1000 ppb, T₄: Control] Error bar indicates SD of 3 replicates

Soluble solid content (SSC)

Although, the SSC in the mango fruits has increased with the increase in storage period, reaching maximum on ninth day and then declined slowly towards the end of storage period (Fig. 3a). Irrespective of the storage period, the SSC was maximum (28.67 °B) in the untreated ‘Amrapali’ mango fruits, followed by the fruits which received treatments 1-MCP @ 1000 and 750 ppb (27.88 and 25.83 °B), respectively and minimum SSC (20.11 °B) was observed in the untreated mango fruits towards the end of storage life (Fig. 3a). In case of 1-MCP treated fruits, the SSC increased slowly and steadily up to certain days of storage and thereafter declined gradually while in control (untreated) fruits, the SSC increased initially for few days and thereafter a sharp decline was noticed, indicating rapid

metabolic activity in these fruits. The lower SSC in 1-MCP treated mango fruits might be due to delayed ethylene production and metabolic activities of fruits during storage with concomitant delay in ripening. The suppressed and delayed increase in SSC was reported in mango (Barman 2013), and plum (Sharma et al. 2012b).

Ascorbic acid content (AAC)

The ascorbic acid content of the ‘Amrapali’ mango fruits has shown a sharp increase initially and then a definite declining trend during ripening and storage. The AAC reached maximum (33.07 mg 100 g⁻¹) in the 1-MCP @ 1000 ppm treated mango fruits on the ninth day of storage while it reached maximum (32.21 mg 100 g⁻¹) on sixth day of storage in the untreated (control) mango fruits.

Fig. 5 RT-PCR expression analysis of *MiExpA1* gene in ‘Amrapali’ mango fruits after 4, 24, 48 h, 5 and 7 days of 1-MCP treatment and storage at ambient conditions [Lane 1: 500 ppb, 2: 750 ppb, 3: 1000 ppb, 4: Control]

Towards the end of storage life, lowest ascorbic acid ($19.55 \text{ mg } 100 \text{ g}^{-1}$) content was observed in the untreated mango fruits (Fig. 3b). The increase in vitamin C content in the initial stages of ripening might be due to increase in the synthesis of some metabolic intermediates which promote the synthesis of precursors of vitamin C. The decrease in vitamin C during the later stages of ripening is due to its oxidation to dehydroascorbic acid. Similar trend of increase and then decrease in the vitamin C content was reported by Gupta et al. (1979) and Itoo et al. (1980) in guava and Broughton and Wong (1979) in sapota. The retention of higher vitamin C contents in 1-MCP treated mango fruits might be due to accumulation of active oxygen species (AOS) in fruits which in turn may help in preventing loss of vitamin C.

Gene expression studies

1-MCP treatment at all the concentrations had significantly repressed the expression of the *MiExpA1* gene compared to its induction in the untreated mango fruits. However, the induction was significantly low in the fruits exposed to 1000 ppb 1-MCP (Figs. 4 and 5) which was also in concurrence with the retention of higher firmness in the same treatment (Table 1). The gene ‘*expansin*’ was found to be repressed by 80, 94 and 94 per cent after 4 h of treatment, with 1-MCP (500, 750 and 1000 ppb), respectively. After 24 and 48 h, the repression of the gene was (49, 53 and 58%) and (58, 59 and 70%), respectively (Figs. 4 and 5). After 5 days of storage, the expression of the ‘*expansin*’ gene had suddenly induced by 651, 131 and 1318%, respectively for the fruits treated with 1-MCP (500, 750 and 1000 ppb). The expression of *expansin* transcripts was found to be induced after 5 days of treatment and continued thereafter. This might be due to regulation of *expansin*

gene expression by ethylene and its inhibition by 1-MCP. After 5 days of treatment the effect of 1-MCP on ethylene production might be reduced due to regeneration of new ethylene binding sites over the fruit surface and expansin protein activity might be reinitiated. The effective concentration of 1-MCP might vary with commodity, time, temperature and method of application (Blankenship and Dole 2003). Here, in this case, the concentrations 500 and 1000 ppb were found to lose their effectiveness little earlier in downregulating the *expansin* gene compared to the 750 ppb concentration and hence their expression levels were promoted clearly after 5 days. Similar repression of this gene at the transcriptional and translational level by 1-MCP has also been reported by Sane et al. (2005) in the mango cv. Dashehari. Since 1-MCP delays ripening through blocking of ethylene receptors, a decrease in *MiExpA1* transcript levels in the treated fruit demonstrates that it is an ethylene and ripening related ‘*expansin*’ which can be used as a candidate gene for manipulation of softening in mango via recombinant DNA technology.

Conclusion

It was concluded that exposure of ‘Amrapali’ mango fruits to 1-methylcyclopropane extended the economic shelf life at ambient conditions ($30 \pm 5^\circ \text{C}$ and $50 \pm 5\% \text{ RH}$) by 3 days compared to the untreated control fruits. 1-MCP (1000 ppm) retained textural firmness and delayed the climacteric ripening process to a greater extent compared to other concentrations. The fruit quality assessed at different intervals of storage, revealed that, all the quality parameters viz. physiological loss in weight, soluble solids concentrates, total antioxidant activity and ascorbic acid content were found to be influenced and improved significantly with 1-MCP treatment up to twelfth day of storage. The gene expression study has revealed that 1-MCP could significantly repress the softening related *expansin* gene through control of ripening process and the present study opens up the potential for biotechnological manipulation of softening in mango fruits.

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