1-MCP application suppresses ethylene biosynthesis and retards fruit softening during cold storage of 'Tegan Blue' Japanese plum

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Abstract

Plum is a highly perishable fruit and postharvest fruit softening limits its cold storage life. To investigate the role of 1-methylcyclopropene (1-MCP) in ethylene biosynthesis and fruit softening during cold storage, Japanese plum (*Prunus salicina* Lindl. cv. Tegan Blue) as harvested at commercial fruit maturity and exposed to 1-MCP (0.0, 0.5, 1.0 and 2.0 μL L^{-1}) at 20 ± 1°C for 24 h. Following 1-MCP treatments, fruit were stored at 0 ± 1°C and 90 ± 5% RH for 0, 3 and 6 weeks. 1-MCP treatments significantly reduced endogenous ethylene production in plum fruit after 3 and 6 weeks of cold storage when compared to untreated fruit. Fruit treated with 1-MCP (1.0 and 2.0 μL L^{-1}) was more firm (31% and 33.5% respectively) when compared untreated fruit. Activities of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) enzymes during cold storage also decreased in 1-MCP-treated fruit skin and pulp tissues and 1-aminocyclopropane-1-carboxylic acid (ACC) content was not detected in the skin and pulp tissues of fruit treated with 1.0 and 2.0 μL L^{-1} 1-MCP. Activities of exo-polygalacturonase (exo-PG) and endo-polygalacturonase (endo-PG) enzymes in the fruit skin tissues were not affected by 1-MCP whereas activities of exo-PG and endo-PG enzymes in fruit pulp tissues, and activities of pectin esterase (PE) and endo-1,4-*β*-D-glucanase (EGase) enzymes in both fruit skin and pulp tissues were significantly reduced during cold storage. Activities of ethylene biosynthesis and fruit softening enzymes were concentration dependent, and both were reduced with increased concentrations of 1-MCP. In conclusion, 1-MCP application extend cold storage life of 'Tegan Blue' plum by suppressing ethylene biosynthesis and reducing fruit softening.

Keywords: ACC; ACO; ACS; EGase; Endo-PG; Enzymes; Exo-PG; PE; *Prunus salicina* Lindl.

1. Introduction

Ripening in climacteric fruit is regulated by ethylene, which triggers the physiological and biochemical changes related to fruit ripening, such as fruit skin colour, sugar and organic acid metabolism and fruit softening [1]. During the process of fruit ripening, softening reduces storage- and shelf-life of plum. Plum is a highly perishable fruit and becomes over-ripe very quickly. Storage life is limited even at low temperature. Due to high susceptibility to physiological disorders at low temperature Japanese plum fruit can be stored at low temperatures for only 3 to 5 weeks [2].

Some of the strategies reported to retard metabolic changes and to improve storage life and post-storage quality in plum, includes: lowering the storage temperature, modifying air composition and packaging [3], inhibition of ethylene production [4], exogenous application of calcium [5] and edible coatings [2]. However, the information is sporadic and inconclusive.

Postharvest physiochemical changes associated with fruit ripening in plum include fruit softening, changes in fruit skin colour, fruit texture, aroma volatiles, and flavour, and they are controlled by endogenous ethylene production [6]. 1-Methylcyclopropene (1-MCP) has been reported to be a non-toxic antagonist of ethylene action [7] that blocks the physiological action of ethylene [8]. Applications of 1-MCP to delay fruit ripening and extend the storage life have been extensively

reported in both climacteric and non-climacteric fruit [9], including some cultivars of plum [10, 11].

The inhibition of ethylene biosynthesis by 1-MCP has been attributed to reduction in the activities of 1-aminocyclopropane-1-carboxylic acid synthase (ACS, EC 4.4.1.14) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO, EC 4.4.17.4) and their respective genes [12]. 1-MCP-treated nectarines exhibited lower ACS, *ACO1* and *ACO2* transcript accumulation than untreated fruit at ambient temperature [13]. Recently, reduction in the activities of ACS and ACO, and 1-aminocyclopropane-1-carboxylic acid (ACC) content during fruit ripening at ambient temperature has been reported in 1-MCP-treated plum skin and pulp tissues [4]. However, the effects of 1-MCP application on ethylene biosynthesis during cold storage have not yet been studied in plum.

Postharvest fruit softening is associated with activities of cell wall hydrolysis enzymes [14]. Polygalacturonase (PG, EC 3.2.1.15) has been reported as a key enzyme involved in fruit softening [15], but pectin methyl esterase (EC 3.1.1.11) [16] and cellulase (EC 3.1.1.4) [17] are also involved. Changes in the activities of cell wall softening enzymes have been investigated in avocado and pear [18, 19]. PG is involved in the hydrolytic cleavage of α-(1-4) galacturonan linkages [20], and is responsible for pectin disassembly during fruit softening process [21]. 1-MCP has been reported to retard fruit softening during fruit ripening in climacteric fruit [9] through inhibiting the activities of cell wall hydrolytic enzymes [19]. During fruit ripening at ambient temperature, reduction in the activities of fruit softening enzymes such as exo-polygalacturonase (exo-PG, EC 3.2.1.67), endo-polygalacturonase (endo-PG, EC 3.2.1.15), pectin esterase (PE EC 3.1.1.11) and endo-1,4-*β*-D-glucanase

(EGase, EC 3.1.1.4) have been reported in 1-MCP-treated plum fruit [4], but no information is available on the effects of 1-MCP on fruit softening or the activities of fruit softening enzymes during cold storage.

The aims of the present study were to: 1) investigate the role of exogenous application of 1-MCP in regulation of ethylene biosynthesis and activities ethylene biosynthesis enzymes (ACS, ACO), and ACC content, and 2) fruit firmness and activities of fruit softening enzymes (exo-PG, endo-PG, PE and EGase) in skin and pulp tissues of 'Tegan Blue' plum during cold storage.

2 Materials and methods

2.1. Plant materials

Uniform Japanese plum (*Prunus salicina* Lindl. cv. Tegan Blue) trees grafted on myrobalan (*Prunus cerasifera* Ehrh.) rootstock at Casuarina Valley Orchard, Manjimup (lat. 34^{o}15´S: long. 116^{o}09´E), in the South West region of Western Australia, were selected for the experiment. The 17-year old trees were planted in a north-south row direction, with 4.5 m between rows and 2 m within the rows and trained on a palmette system. Plum fruit at commercial harvest maturity (TSS 16.3 ± 0.9% and firmness 60.3 ± 2.6N) of uniform size, free from visual symptoms of any disease or blemishes were harvested. The bulked fruit were transported to the laboratory immediately after harvest. The fruit were randomly selected for different treatments.

2.2. 1-MCP application and experimental design

The fruit were kept in hermetically sealed plastic drums (68 L), and 1-MCP was injected into the drums through a rubber septum to obtain concentrations of 0.0, 0.5,

1.0 or 2.0 μL L^{-1}. 1-MCP gas was obtained from EthylBlock™ powder (active ingredient 0.43% 1-MCP, BioTechnologies for Horticulture Inc., Waterboro, SC, USA) following the method of Lalel et al. [22]. Fruit were treated with 1-MCP for 24 h at 20 ± 1°C.

All treatments were replicated three times with eight fruit as an experimental unit for each parameter studied. Following 1-MCP treatments fruit were stored for 0, 3 and 6 weeks at 0 ± 1°C and 90 ± 5% RH. At the end of each storage period, measurements were made of ethylene production, fruit firmness, activities of ethylene biosynthesis enzymes (ACS and ACO), ACC content and activities of exo-PG, endo-PG, PE and EGase.

2.3. Determination of ethylene production

Two fruit representing a replicate were sealed in an airtight jar (1,000 ml) fitted with a rubber septum for 1 hour at room temperature (20 ± 1°C). One ml of headspace gas sample was injected into a GC-FID (Agilent Technologies, 6890 N Network GC system, Palo Alto, CA, USA) and ethylene production (μmol kg^{-1} h^{-1}) was determined as reported by Khan and Singh [4].

2.4. Ethylene biosynthesis enzymes and ACC content in fruit skin and pulp tissues

Activities of ethylene biosynthesis enzymes (ACS and ACO) and ACC content were determined from fruit skin and pulp tissues using the method reported by Khan and Singh [4].

2.5. Fruit firmness

Fruit firmness was determined using an electronic pressure tester (model EPT-1 pressure tester, Lake City Technical Products Inc., Kelowna, BC, Canada) fitted with

an 8 mm tip. A small slice of fruit skin was removed from each side of a fruit and the firmness was recorded and expressed as newtons (N).

2.6. Determination of fruit softening enzymes in fruit skin and pulp

Activities of fruit softening enzymes, including exo-PG, endo-PG, EGase and PE from fruit skin and pulp tissues were determined by following the modified method for plum fruit as reported earlier by Khan and Singh [4].

2.7. Protein determination

Protein content from fruit skin or pulp tissue were determined using the method of Bradford [23].

2.8. Statistical Analysis

Data were subjected to analysis of variance (ANOVA), using Genstat 9.1 release (Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). Data using two-factor (1-MCP concentrations and storage period) factorial design were analysed.

Assumptions of ANOVA were checked, and least significant differences (Fisher's LSD) were calculated at $P \leq 0.05$ following significant F tests. Orthogonal polynomial contrasts were performed within the ANOVA to obtained trends and to relate the different concentrations of 1-MCP to different mean responses at $P \leq 0.05$. Pearson correlation coefficients were calculated using SPSS v.14.0 for windows, USA, to determine the association between variables.

3. Results

3.1. Ethylene production

Postharvest application of 1-MCP significantly reduced ethylene production after cold storage for 3 and 6 weeks (Fig. 1). Reduction in ethylene production with various concentrations of 1-MCP showed a linear response and the reduction was more pronounced with higher 1-MCP concentrations (1.0 and 2.0 μL L^{-1}).

3.2. Ethylene biosynthesis enzymes and ACC content in fruit skin and pulp tissues

Activities of ACS were significantly reduced in 1-MCP-treated fruit skin tissues, as compared to untreated fruit (Fig. 2A). 1-MCP (1.0 and 2.0 μL L^{-1}) treatments completely inhibited the activities of ACS enzyme in fruit skin tissues, whereas untreated fruit exhibited 24% and 49% higher ACS activities in skin tissues after 3 and 6 weeks of storage, as compared with those with zero weeks storage. Similarly, after 3 and 6 weeks of storage ACS activities in pulp tissues of untreated fruit were 33.3% and 39.7% higher than 2.0 μL L^{-1} 1-MCP-treated fruit (Fig. 2B). Mean ACS activities of skin tissues were 6-fold higher than in pulp tissues.

1-MCP reduced the activities of ACO in fruit skin and pulp tissues, when compared with untreated fruit (Fig. 3). Fruit treated with a higher concentration of 1-MCP (2.0 μL L^{-1}) produced no change in ACO activities, even after 6 weeks of storage whereas 0.5 and 1.0 μL L^{-1} 1-MCP-treated fruit skin tissues showed 15.1% and 36.2% reduction in ACO activities. The activities of ACO in the pulp tissues of 1-MCP (1.0 and 2.0 μL L^{-1}) treated fruit were reduced by 28.6% when compared with the controls (Fig. 3B). ACO activity showed a linear response to various concentrations of 1-MCP applied. Mean activities of ACO were 10-fold higher in skin tissues than in pulp tissues.

Changes in ACC content in 1-MCP-treated fruit skin tissues were completely inhibited during the storage period irrespective of concentrations of 1-MCP applied

(Fig. 4A) whereas skin of untreated fruit showed 78.7% and 85.2% increase in ACC content after 3 and 6 weeks of storage, respectively. Postharvest 1-MCP application also reduced the ACC content in pulp tissues (Fig. 4B). After 6 weeks of storage, ACC content in the pulp tissues of 1-MCP (1.0 and 2.0 μL L^{-1}) treated fruit was reduced by 41.4% and 51.3%, respectively as compared with 0.5 μL L^{-1} 1-MCP-treated and untreated fruit, respectively. Skin tissues exhibited 2.6-fold higher mean ACC content than pulp tissues. Changes in the ACC content in fruit skin and pulp tissues showed a linear response to different concentrations of 1-MCP applied.

3.3. Fruit firmness

1-MCP application significantly delayed fruit softening during storage. Fruit treated with 1-MCP were more firm than untreated fruit after 3 and 6 weeks of cold storage (Fig. 5).

3.4. Fruit softening enzymes in fruit skin and pulp

The activities of exo-PG enzyme in fruit skin tissues were not influenced by 1-MCP treatments or cold storage period (Fig. 6A). However, in pulp tissues, activities of exo-PG enzyme increased during the storage period as the concentrations of 1-MCP applied decreased (Fig. 6B). After 6 weeks of storage, pulp tissues of untreated fruit exhibited 19.6, 23.8 and 30.3% higher exo-PG activities as compared to 0.5, 1.0 and 2.0 μL L^{-1} 1-MCP-treated fruit pulp tissues respectively. 1-MCP treatment did not significantly affect the activities of endo-PG in fruit skin tissues (Fig. 7A). After 3 weeks of cold storage, 2.0 μL L^{-1} 1-MCP-treated fruit showed reduced activities of endo-PG enzymes in pulp tissues, as compared to the control fruit (Fig. 7B). After 6 weeks of cold storage, activities of exo-PG in pulp tissues were significantly

decreased by 1.0 and 2.0 μL L^{-1} 1-MCP treatments as compared to other treatments (Fig. 7B).

Activities of PE in control fruit skin and pulp tissues increased with extended storage time, whilst in 1-MCP treated fruit skin and pulp tissues, PE activities were lower during storage with increase in concentration of 1-MCP applied (Fig. 8). 1-MCP-treated fruit skin tissues exhibited 38.5% reduction in activities of PE after 3 and 6 weeks of storage compared with untreated fruit (Fig. 8A). After 3 weeks of storage, untreated fruit pulp tissues exhibited 25.7% and 42.9% higher PE activity than fruit treated with 1.0 and 2.0 μL L^{-1} 1-MCP (Fig. 8B). Pulp tissues showed 1.5-fold higher mean PE activity than the skin tissues.

1-MCP treatment significantly reduced the activities of EGase enzymes in skin and pulp tissues during cold storage (Fig. 9). Mean EGase activities were about 2-fold higher in pulp tissues in contrast to fruit skin tissues. EGase activities in 1-MCP (2.0 μL L^{-1}) treated fruit skin tissues were reduced by 24.7% and 31.4% after 3 and 6 weeks of storage when compared with the control fruit. Similarly, 2.0 μL L^{-1} 1-MCP-treated fruit pulp tissues exhibited 25.4% and 34.7% reduction in EGase activities after 3 and 6 weeks cold storage when compared with activities in the pulp of untreated fruit. Activities of fruit softening enzymes showed a significant linear response to different concentrations of 1-MCP applied.

3.5. Relationship between ethylene biosynthesis, and fruit softening enzymes, and in fruit softening variables

Fruit firmness showed significant ($P \leq 0.01$) negative correlations with ACC content (r = -0.461, -0.908), and activities of ACS (r = -0.702, -0.747) and ACO (r = -0.839, -656) enzymes in fruit skin and pulp tissues.

Fruit firmness showed a significant ($P \leq 0.01$) negative correlations with exo-PG activities in pulp tissues (r = -0.831), while ethylene production regulated with 1-MCP application showed a significant ($P \leq 0.01$) positive correlation with exo-PG activities (r = 0.43, 0.796) in skin and pulp tissues respectively. PE activities in skin and pulp tissues of 1-MCP-treated fruit showed significant ($P \leq 0.01$) negative correlations (r = -0.927, -0.845) with fruit firmness respectively, while the activities of PE in the skin and pulp tissues of 1-MCP-treated fruit showed significant ($P \leq 0.01$) positive correlations with ethylene production (r = 0.745, 0.674) respectively. There were no significant correlations between ethylene production and endo-PG and EGase activities in fruit skin and pulp tissues.

4. Discussion

The reduction in ethylene production during cold temperature storage with 1-MCP application may be ascribed to the ability of 1-MCP to interact with ethylene receptors and its competition with ethylene for binding sites [24] or to the ability of 1-MCP to interfere with autocatalytic ethylene production [22], as ethylene binding sites are irreversibly blocked by 1-MCP [8]. Reduction in ethylene production in 1-MCP-treated fruit during storage may also be attributed to the reduced activities of ethylene biosynthesis enzymes and ACC content during cold storage (Figs 1-4). Reduction in endogenous ethylene production by 1-MCP has also been reported in plum stored at low temperature [10, 11].

1-MCP reduced ethylene production is attributable to the ability of 1-MCP to bind with ethylene receptors and consequently reduce the normal increase in the activities of ACS and ACO enzymes during ripening [25]. Reduced activities of ACS and ACO enzymes have been reported in 1-MCP-treated plum during fruit ripening [4]. In

apple, pear and fig, inhibition of ethylene production was also accompanied by reduced expression of ACS and ACO transcripts [26-28], which suggests that the reduction in ethylene production by 1-MCP is regulated at the gene level. Similarly, inhibition of ethylene production in peach fruit was associated with reduced activities of ACO and with reduction in *PP-ACO1* and *PP-ACO2* transcript accumulation [12]. 1-MCP application to avocado fruit at the pre-climacteric stage, and at the onset of the climacteric stage inhibited ACS and ACO activities and the transcription of *PA-ACS1*, and suppressed *PA-ACO* and *PA-ERS1* mRNAs to trace levels [28]. A significant delay in the induction of ethylene biosynthesis and *PP-ACSI* and *PP-ACOI* genes has also been reported in 1-MCP-treated pear [26].

Our experimental data show that 1-MCP application completely inhibited the ACC content in the skin tissues irrespective of concentrations of 1-MCP applied. Pulp tissues treated with higher 1-MCP (1.0 and 2.0 μL L^{-1}) had dramatically lower ACC content during storage than did untreated tissues. The reduction in the ACC content in skin and pulp tissues may be due to reduced activities of ACS in the skin as well as in the pulp during cold storage, or by conversion of ACC to malonyl or glutamylamino derivatives instead of ethylene. ACC malonylation has been reported to a contributing factor in the limited production of ACC [29] in pre-climacteric fruit.

Application of higher concentrations of 1-MCP to 'Tegan Blue' plum maintained significantly firmer fruit during storage. The reduction in plum fruit softening with 1-MCP treatment may be attributed to suppressed ethylene production, and consequently, reduced the fruit softening. Reduction in the activities of fruit softening enzymes was seen in an earlier study [4] and the maintenance of fruit firmness following 1-MCP treatment was found to be negatively correlated with activities

ethylene biosynthesis enzymes and fruit softening enzymes. Reduction in fruit softening by 1-MCP has also been reported in peach [30] and plum [10] during cold storage.

Postharvest 1-MCP application significantly reduced exo-PG and endo-PG activities in pulp tissues during cold storage (Figs 6B and 7B). Similarly, reduced activities of PG enzymes have been reported in 1-MCP treated peach and persimmon fruit [31, 32]. Delayed fruit softening is also considered to be associated with the delayed increase in the soluble pectin concentrations [33]. Activities of PE enzymes in untreated fruit increased with increase in the storage period, whereas 1-MCP application significantly reduced the activities of PE enzymes both in skin and pulp tissues (Fig. 8). It has been widely recognised that during fruit softening, PE removes the C-6 position of galacturonic acid polymers which then enables PG to depolymerise the de-esterified polygalacturonide chain [34]. The increase in the endo-PG activity followed the rise in PE activity in both treated and untreated fruit skin and pulp tissues, which suggests that PE may be the prerequisite for PG activities during fruit softening [35]. It may also be argued that reduction in the activities of these fruit softening enzymes may be due to reduction in endogenous ethylene production by 1-MCP treatment as the activities of exo-PG and PE enzymes were significantly correlated with endogenous ethylene production. Similar changes in PE have been reported in 1-MCP-treated persimmon [32] and reduced PG and PE activities have also been observed in treated avocado fruit [19]. Dramatic increase in EGase activity, protein and mRNA levels have been observed during ripening of pear fruit [18]. Our experimental data suggest that 1-MCP significantly reduced the activities of EGase enzymes in skin and pulp tissues during cold storage. A similar reduction in activities of EGase were also found in 1-MCP-treated avocado [19].

In conclusion, 1-MCP (1.0 μL L^{-1}) application to 'Tegan Blue' plum suppressed ethylene production , activities of ethylene biosynthesis enzymes, fruit softening, and activities of fruit softening enzymes in fruit skin and pulp tissues during cold storage. 1-MCP-treated fruit also showed differential activity of fruit softening and ethylene biosynthetic enzymes in the skin and pulp tissues, which indicates that further investigations are needed on expression of genes encoding these enzymes.

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Legends for figures

Fig. 1.

Effects of different concentrations of 1-MCP on the ethylene production of 'Tegan Blue' plum stored for 0, 3 and 6 weeks. Vertical bars represent S.E. of means, n = 3.

Fig. 2.

Effects of different concentrations of 1-MCP on the activities of ACS as ACC production on a mg protein basis (pmol ACC mg^{-1} protein h^{-1}) enzyme in fruit skin (A) and pulp (B) tissues of 'Tegan Blue' plum stored for 0, 3 and 6 weeks. Vertical bars represent S.E. of means, n = 3.

Fig. 3.

Effects of different concentrations of 1-MCP on the activities of ACO as ethylene production on a mg protein basis (nmol C_2H_4 mg^{-1} protein h^{-1}) enzyme in fruit skin (A) and pulp (B) tissues of 'Tegan Blue' plum stored for 0, 3 and 6 weeks. Vertical bars represent S.E. of means, n = 3.

Fig. 4.

Effects of different concentrations of 1-MCP on the ACC content (pmol g^{-1} FW) in fruit skin (A) and pulp (B) tissues of 'Tegan Blue' plum stored for 0, 3 and 6 weeks. Vertical bars represent S.E. of means, n = 3.

Fig. 5.

Effects of different concentrations of 1-MCP on the fruit firmness (N) of 'Tegan Blue' plum stored for 0, 3 and 6 weeks. Vertical bars represent S.E. of means, n = 24 (8 fruits x 3 replications).

Fig. 6.

Effects of different concentrations of 1-MCP on the activities of exo-PG (μg galacturonic acid mg^{-1} protein h^{-1}) enzyme in fruit skin (A) and pulp (B) tissues of 'Tegan Blue' plum stored for 0, 3 and 6 weeks. Vertical bars represent S.E. of means, n = 3.

Fig. 7.

Effects of different concentrations of 1-MCP application on the activities of endo-PG (viscosity changes mg^{-1} protein h^{-1}) enzyme in fruit skin (A) and pulp (B) tissues of 'Tegan Blue' plum stored for 0, 3 and 6 weeks. Vertical bars represent S.E. of means, n = 3.

Fig. 8.

Effects of different concentrations of 1-MCP application on the activities of PE (mM NaOH mg^{-1} protein h^{-1}) enzyme in fruit skin (A) and pulp (B) tissues of 'Tegan Blue' plum stored for 0, 3 and 6 weeks. Vertical bars represent S.E. of means, n = 3.

Fig. 9.

Effects of different concentrations of 1-MCP application on the activities of EGase (viscosity changes mg^{-1} protein h^{-1}) enzyme in fruit skin (A) and pulp (B) tissues of 'Tegan Blue' plum stored for 0, 3 and 6 weeks. Vertical bars represent S.E. of means, N = 3.

Figure 1 *A. S. Khan, Z. Singh*

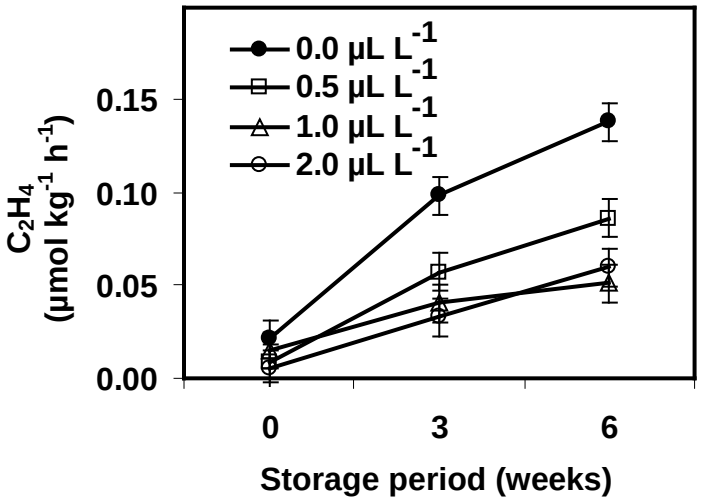

Figure 2 *A. S. Khan, Z. Singh*

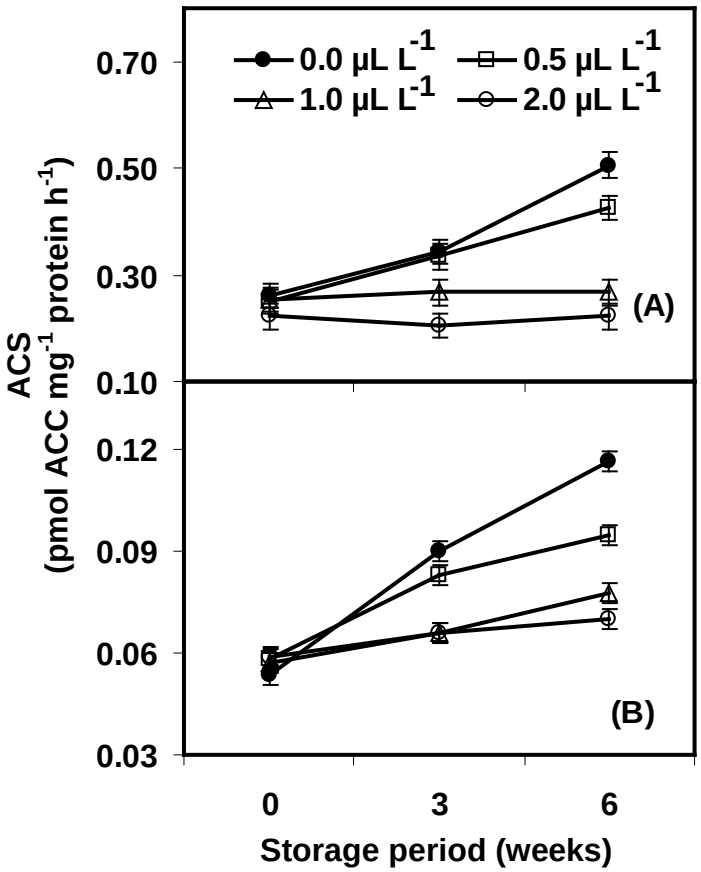

Figure 3 *A. S. Khan, Z. Singh*

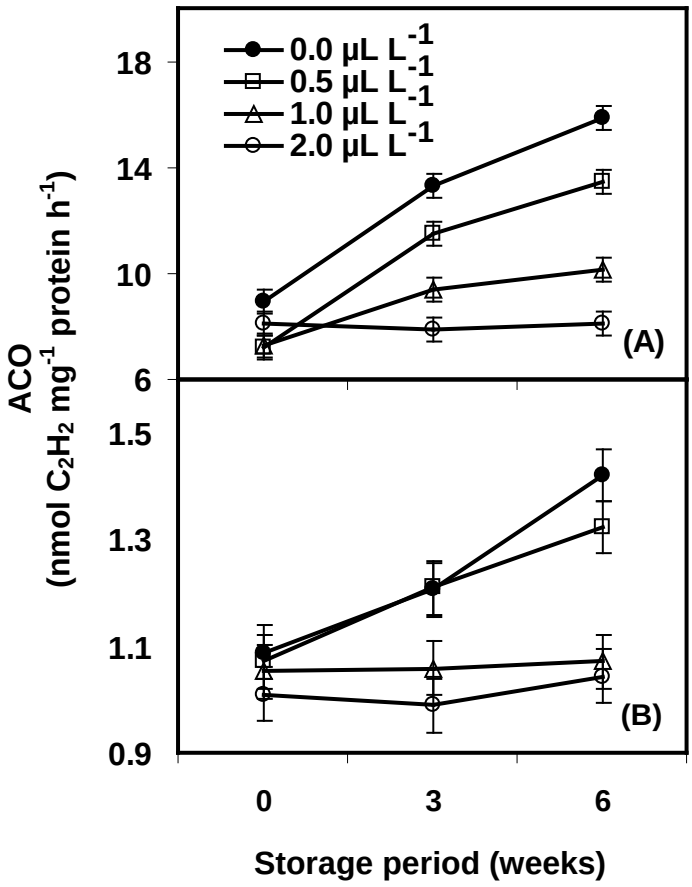

Figure 4 *A. S. Khan, Z. Singh*

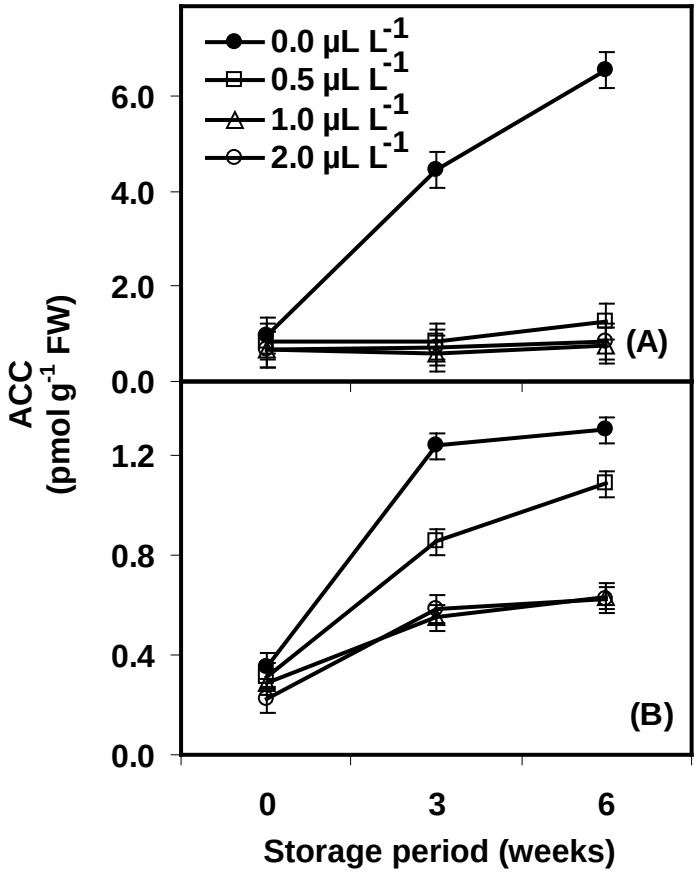

Figure 5 *A. S. Khan, Z. Singh*

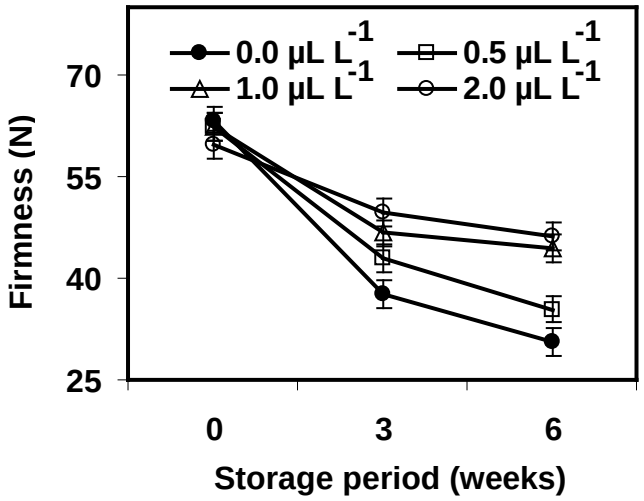

Figure 6 *A. S. Khan, Z. Singh*

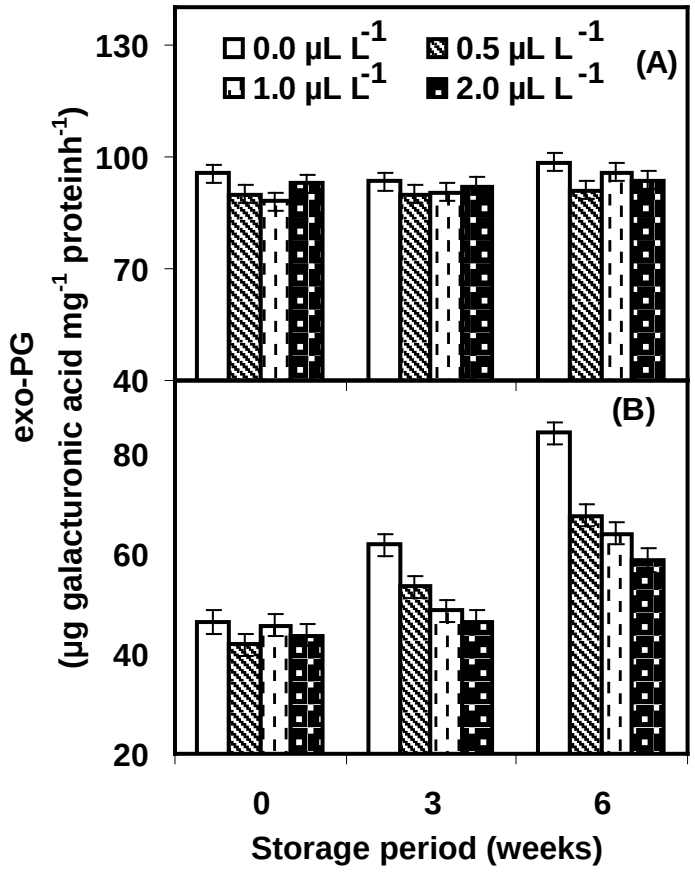

Figure 7 *A. S. Khan, Z. Singh*

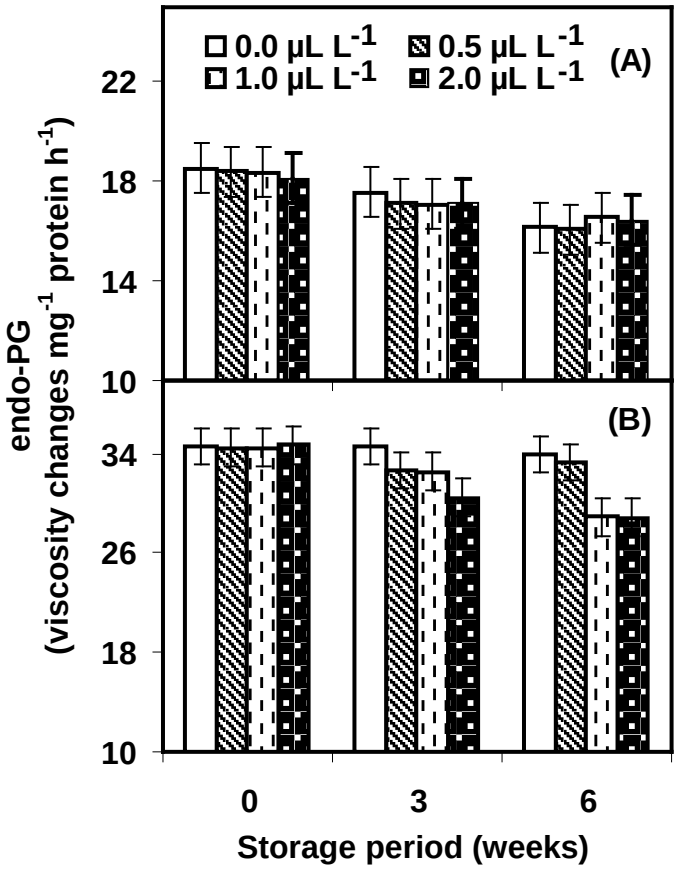

Figure 8 *A. S. Khan, Z. Singh*

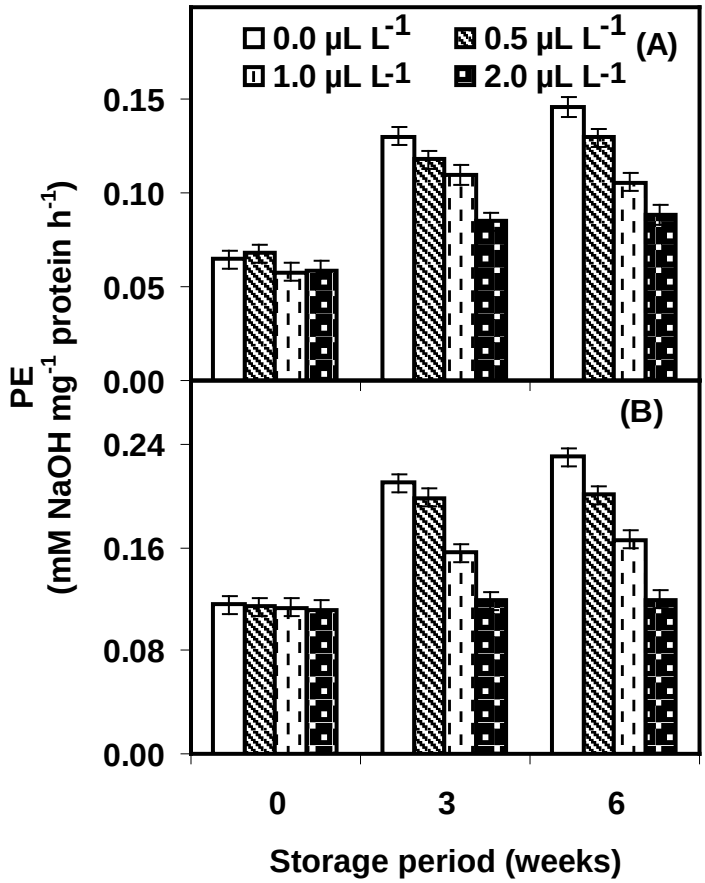

Figure 9 *A. S. Khan, Z. Singh*